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Low coherence optical methods for express non-
invasive bio-medical diagnostics

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invasive bio-medical diagnostics

Supervisor: *Dr. Igor Meglinski, Cranfield Health, Cranfield University*

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To My Family

Abstract

Coherence is the fundamental property of optical radiation. Methods based on this characteristic are employed in different areas of research even in NASA and European space agency. However, coherence properties of light can be successfully applied in life sciences for the purposes of diagnostics and treatment. The subject of current research is the development and improvement of the quantitative performance of non-invasive low coherence optical diagnostic which is a novel modern biomedical technique uniquely suited for the measurements of particles distribution within the topical layers of biological tissues and other complex media, such as polymers, colloids, composite materials, etc. This approach is based on the illumination of a medium by optical radiation with a low coherence both spatial and temporal, and analysis of the back-scattering signal. Recently introduced this technique is not currently used. The main difficulty in the developing of practical low-coherence systems in biomedical diagnostics is associated with the complexity of biotissues structure. For example, the human skin has very complex structure and consists with several layers of cells with unique optical properties in each layer. External boundary and internal boundaries between layers are rough and wavy. Thus, the most accurate description of optical radiation propagation is extremely complicated to achieve and can be performed only as a computer simulation. All further investigations of the optical methods of biotissues diagnostics can be enhanced using such computer models.

Aims and objectives

The aim of this research is to investigate theoretical model of low coherent radiation propagation and localization within the random media and to develop the experimental system suitable for the different biomedical applications. In the current research the Monte Carlo (MC) technique was applied for the purposes of computer simulation of low-coherent optical propagation within the skin and optimization of the parameters of existing systems. The MC method makes it possible to give a realistic picture of the processes involved into the light-tissue interaction. Although the modelling with the MC is quite time consuming, it is the most efficient technique to describe the radiation propagation in the scattering turbid medium. Existing MC code was improved with coherence and polarization effects, which significantly increasing the accuracy of the modelling. MC model was expanded with the new computational method that is able to consider all the scattering orders within a detected OCT signal. MC model was used also for the purpose of visualization of the inhomogeneity in turbid medium with the help of artificial neural network.

In addition, low-coherence optical techniques, such as optical coherence tomography (OCT), its extension Doppler OCT and confocal laser microscopy (CLSM) were applied for the purposes of bio-tissues diagnostics and imaging of their internal structure. The conventional OCT was applied for the purpose of scaffold structure imaging. At the same time Doppler OCT was applied for the measurements of spatial fluid flow velocity distribution within this complex scaffold structure. Obtained results make it possible to evaluate the shear stress within the scaffold in a particular area and optimize input flow rate consequently. CLSM was employed for the investigations of image contrasting with gold nanoparticles.

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Table of Contents

ABSTRACT	II
AIMS AND OBJECTIVES.....	III
ACKNOWLEDGEMENTS	IV
TABLE OF CONTENTS	V
LIST OF FIGURES.....	VIII
LIST OF ABBREVIATIONS.....	XI
LIST OF PUBLICATIONS AND CONFERENCES	XIII
PUBLICATIONS IN JOURNALS	XIII
CONFERENCE PROCEEDINGS	XIII
CONFERENCE PRESENTATIONS	XIV
CHAPTER 1. INTRODUCTION.....	1
CHAPTER 2. LIGHT-TISSUE INTERACTION.....	9
2.1 ELECTROMAGNETIC WAVE THEORY OF LIGHT	9
2.2 EFFECTS OF INTERACTION BETWEEN RADIATION AND MEDIUM	15
2.2.1 Absorption	15
2.2.1.1 Aspects of absorption in the context of quantum theory	17
2.2.2 Absorption within biomedical tissues	19
2.2.3 Refraction and reflection within the medium.....	21
2.2.4 Scattering of light inside bio-tissues	24
2.2.4.1 Scattering cross- section, coefficient, and mean free path length.....	27
2.2.4.2 Scattering phase function and anisotropy factor.....	28
2.2.4.3 Mie scattering	31
2.3 SUMMARY	34
CHAPTER 3. RADIATIVE TRANSFER THEORY	35
3.1 TRANSPORT THEORY	37
3.1.1 Photon distribution function and photon power	37
3.1.2 Radiance	37
3.1.3 Fluence rate.....	38
3.1.4 Net flux.....	38

3.1.5 Radiation transport equation.....	40
3.2 DIFFUSION THEORY	43
3.2.1 Time-domain and point source solutions.....	47
3.2.2 Frequency domain solutions.....	51
3.3 SUMMARY	52
CHAPTER 4. MONTE CARLO SIMULATION AS A COMPUTER METHOD OF RADIATIVE TRANSFER EQUATION SOLUTION.....	53
4.1 PRINCIPLES OF MONTE CARLO METHOD	53
4.2 ASSUMPTIONS USED IN THE MODELLING	57
4.3 SUMMARY	63
CHAPTER 5. LOW-COHERENCE OPTICAL TECHNIQUES AND THEIR APPLICATION	64
5.1 OPTICAL COHERENCE TOMOGRAPHY (OCT)	64
5.1.1. Main characteristics of OCT	68
5.1.2. Applications of OCT in biomedicine.....	70
5.2 DOPPLER OCT	71
5.2.1 Basic principles of Doppler OCT imaging	75
5.2.2 Sensitivity and resolution.....	76
5.3 PRINCIPLES OF CONFOCAL LASER SCANNING MICROSCOPY (CLSM).....	79
5.3.1 Basic concepts of CLSM	80
5.3.2 Advantages and Disadvantages of Confocal Microscopy.....	84
5.4 SUMMARY	85
CHAPTER 6. VALIDATION, IMPROVEMENT AND DEVELOPMENT OF MONTE CARLO METHOD.....	86
6.1 VALIDATION: IMPACT OF PROBE GEOMETRY ON THE DETECTING FIELD OF VIEW	86
6.2 IMPROVEMENT: NEW COMPUTATIONAL MODEL FOR SIMULATION OF POLARIZED LIGHT PROPAGATION WITHIN BIOLOGICAL TISSUES	89
6.3 DEVELOPMENT: SIMULATION OF POLARIZATION-SENSITIVE OPTICAL COHERENCE TOMOGRAPHY IMAGES BY A MONTE CARLO METHOD.....	92
6.3.1 Monte Carlo method in case of polarization	93
6.3.2 Results of the modelling and discussion	96
6.4 DEVELOPMENT: IMAGE RECONSTRUCTION OF THE LOCATION OF MACRO-INHOMOGENEITY IN RANDOM TURBID MEDIUM BY USING ARTIFICIAL NEURAL NETWORKS	99

CHAPTER 7. APPLICATION AND IMPROVEMENT OF LOW-COHERENCE METHODS OF TISSUE DIAGNOSTICS.....	102
7.1 APPLICATION OF OPTICAL COHERENCE TOMOGRAPHY FOR IMAGING OF SCAFFOLD STRUCTURE AND MICRO-FLOWS CHARACTERIZATION	102
7.2 APPLICATION OF GOLD NANOPARTICLES AS CONTRAST AGENTS IN CONFOCAL LASER SCANNING MICROSCOPY	107
7.2.1 <i>Optical properties of gold nanoparticles</i>	109
7.2.2 <i>Description of the experiment</i>	110
7.2.3 <i>Results</i>	112
CONCLUSIONS.....	115
BIBLIOGRAPHY	119

List of figures

Figure 1.1	<i>X-ray, MRI and CT images of human tissues</i>	<i>p.2</i>
Figure 1.2	<i>Imaging of human skin with blood vessels</i>	<i>p.5</i>
Figure 2.1	<i>Electromagnetic radiation spectrum</i>	<i>p.9</i>
Figure 2.2	<i>Exponentially decreasing of light intensity due to the absorption</i>	<i>p.16</i>
Figure 2.3	<i>Jablonski diagram</i>	<i>p.18</i>
Figure 2.4	<i>Stimulated emission</i>	<i>p.19</i>
Figure 2.5	<i>Absorption spectra of some important chromophores in human skin tissue, as function of wavelength</i>	<i>p.20</i>
Figure 2.6	<i>Light incidents to the surface of a medium</i>	<i>p.22</i>
Figure 2.7	<i>Light travelling from one medium into another</i>	<i>p.23</i>
Figure 2.8	<i>Scattering by a discrete particle</i>	<i>p.25</i>
Figure 2.9	<i>Schematic molecular energy level for diatomic molecules</i>	<i>p.26</i>
Figure 2.10	<i>Raman and Rayleigh scattering</i>	<i>p.27</i>
Figure 2.11	<i>A scattering event causes a deflection at angle θ from the original direction $\hat{s}$ to the direction $\hat{s}'$</i>	<i>p.29</i>
Figure 2.12	<i>A series of plots of the Henyey-Greenstein functions for different g-values</i>	<i>p.30</i>
Figure 2.13	<i>A single scattering event considering the incident and the scattered light components</i>	<i>p.32</i>
Figure 3.1	<i>Representation of the three important optical parameter in transport theory</i>	<i>p.39</i>
Figure 3.2	<i>Schematics of the transport equation component terms</i>	<i>p.42</i>
Figure 3.3	<i>Schematics of the two possible representations of the source</i>	<i>p.48</i>
Figure 3.4	<i>Schematic picture showing the principle of an extrapolated boundary</i>	<i>p.49</i>
Figure 4.1	<i>Schematic presentation of Monte Carlo method</i>	<i>p.55</i>
Figure 5.1	<i>Schematic diagram of a Michelson interferometer used in OCT</i>	<i>p.65</i>
Figure 5.2	<i>Effect of the interaction of the probing radiation with a moving</i>	<i>p.74</i>

	<i>particle on the cross-correlation interferometric signal</i>	
Figure 5.3	<i>Human endoscopic D-OCT images of normal esophagus with the five layers indicated</i>	<i>p.78</i>
Figure 5.4	<i>In vivo measurements of sub-cranial cerebral blood flow of a rat</i>	<i>p.79</i>
Figure 5.5	<i>Schematic diagram of the optical pathway and principal components in a laser scanning confocal microscope</i>	<i>p.82</i>
Figure 5.6	<i>Confocal microscope configuration and information flow schematic diagram</i>	<i>p.83</i>
Figure 5.7	<i>Comparison of widefield (a) and laser scanning confocal fluorescence microscopy images (b)</i>	<i>p.84</i>
Figure 6.1	<i>Schematic representation of the experimental set-up, and definition of sampling volume distribution</i>	<i>p.86</i>
Figure 6.2	<i>Sampling volume $J(x, z)$ in a multilayered medium for configurations with: (a) optical fibres; (b) CCD camera; (c) combination of optical fibre and CCD</i>	<i>p.88</i>
Figure 6.3	<i>Intensities of the OCT signals calculated with the suggested (red line) method and the conventional method (black line)</i>	<i>p.91</i>
Figure 6.4	<i>The ratio of the intensities of the OCT signals calculated with the suggested method and the conventional method</i>	<i>p.92</i>
Figure 6.5	<i>Schematic presentation of skin model</i>	<i>p.97</i>
Figure 6.6	<i>Comparison of simulated (a)-(c) and experimental (d)-(f) PS-OCT images</i>	<i>p.98</i>
Figure 6.7	<i>Schematic presentation of the artificial neuron</i>	<i>p.99</i>
Figure 6.8	<i>Intensity distribution of scattering at the surface of scattering medium</i>	<i>p.100</i>
Figure 6.9	<i>Locating of the inhomogeneity in the medium</i>	<i>p.101</i>
Figure 7.1	<i>Schematic diagram of the experimental set-up</i>	<i>p.102</i>
Figure 7.2	<i>Experimental set-up. (a)-reference arm; (b) – sample arm</i>	<i>p.103</i>
Figure 7.3	<i>Image (2x2 mm) of the internal structure of scaffold obtained using the OCT and cross-sectional flow velocity distribution of non-filtered 1% water solution of Intralipid through the scaffold</i>	<i>p.104</i>

Figure 7.4	<i>Microstructure of scaffold obtained with ISI ABT-55 low-vacuum scanning electron microscope. The spatial resolution – 22.7 μm</i>	<i>p.105</i>
Figure 7.5	<i>Cross-sectional flow velocity distribution of filtered water solution of Intralipid through the scaffold and structural image (2×2 mm) of scaffold overlapped with the velocity flow distribution</i>	<i>p.106</i>
Figure 7.6	<i>Schematic diagram of a confocal microscope used in the current research</i>	<i>p.111</i>
Figure 7.7	<i>CLSM images of chicken skin obtained at the focal depth 18 μm</i>	<i>p.112</i>
Figure 7.8	<i>CLSM image of chicken skin obtained at 45 μm focal depth</i>	<i>p.113</i>
Figure 7.9	<i>CLSM images of chicken skin with application of clearing agent</i>	<i>p.114</i>

List of abbreviations

2D	Two-Dimensional
ANN	Artificial Neural Network
CBF	Cerebral Blood Flow
CCD	Charged Coupled Device
CL	Collimating Lens
CLSM	Confocal Laser Scanning Microscopy
CT	Computer Tomography
DLS	Dynamic Light Scattering
DOCT	Doppler Optical Coherence Tomography
DWS	Diffusing Wave Spectroscopy
EM	Electromagnetic
FC	Fibre Coupler
FD-OCT	Fourier Domain Optical Coherence Tomography
FL	Focusing Lens
FWHM	Full Width at Half Maximum
Hb	Deoxygenated Haemoglobin
HbO ₂	Oxygenated Haemoglobin
IC	Internal Conversion
IR	Infrared
ISC	Intersystem Crossing
LCI	Low Coherence Interferometry
MC	Monte Carlo
MRI	Magnetic Resonance Imaging
NIR	Near Infrared
OCT	Optical Coherence Tomography
OD	Optical Density
PBS	Phosphate Buffered Saline
PC	Personal Computer

PD	Photo-detector
PDT	Photodynamic Therapy
PS-OCT	Polarization-Sensitive Optical Coherence Tomography
RA	Reference Arm
RTT	Radiative Transfer Theory
SA	Sample Arm
SI	Système International d'unités
SLD	Superluminescence Diode
SNR	Signal-To-Noise Ratio
SS-OCT	Swept Source Optical Coherence Tomography
ST-FFT	Short-Term Fast Fourier Transform
TD-OCT	Time Domain Optical Coherence Tomography
UV	Ultraviolet
VIS	Visible

List of publications and conferences

Publications in journals

- 1) **B. Veksler**, E. Kobzev, M. Bonesi, I. Meglinski, “Study of scaffold structure and micro-flows in them with optical coherence tomography”, *Optics and Spectroscopy*, in press
- 2) **B. Veksler**, A. Lemelle, I. S. Kozhevnikov, G. G. Akchurin, I. Meglinski, “Improvement of the image contrast in confocal laser scanning microscopy with the help of gold nanoparticles and clearing agents”, *Optics and Spectroscopy*, in press
- 3) A. Lemelle, **B. Veksler**, I. S. Kozhevnikov, G. G. Akchurin, S. A. Piletsky, I. Meglinski, “Application of gold nanoparticles as contrast agents in confocal laser scanning microscopy”, *Laser Physics Letters*, **6**(1), 71-75, 2009.
- 4) **B. A. Veksler**, I. V. Meglinski, “Application of the artificial neural network for reconstructing the internal-structure image of a random medium by spatial characteristics of backscattered optical radiation”, *Quantum Electronics*, **38**(6), 576-579, 2008.
- 5) **B. Veksler**, E. Kobzev, M. Bonesi, I. Meglinski, “Application of optical coherence tomography for imaging of scaffold structure and micro-flows characterization”, *Laser Physics Letters*, **5**(3), 236-240, 2008.
- 6) A. V. Kovaleva, **B. A. Veksler**, I. L. Maksimova, I. V. Meglinski, “Locating of macro-inhomogeneity in random turbid medium by using artificial neural networks”, *Laser Physics Letters*, **4**(5), 337-340, 2007.

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- 1) **B. Veksler**, E. Kobzev, M. Bonesi, I. Meglinski, “Application of optical coherence tomography for imaging of scaffold structure and micro-flows characterization”, in

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- 2) **B. A. Veksler**, I. L. Maksimova and I. V. Meglinski, “Image reconstruction of the location of macro-inhomogeneity in random turbid medium by using artificial neural networks”, in : Biophotonics 2007: Optics in Life Science, Edited by J. Popp and G. von Bally, Proc. SPIE, Vol.6633, 663322 (2007) // ISBN: 9780819467775

Conference presentations

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- 2) **B. Veksler**, E. Kobzev, M. Bonesi, I. Meglinski, “Application of optical coherence tomography for imaging of scaffold structure and micro-flows characterization”, Saratov Fall Meeting (SFM 2008), 23-26 September 2008, Saratov, Russia
- 3) A. Lemelle, **B. Veksler**, I. S. Kozhevnikov, G. G. Akchurin, S. A. Piletsky, I. Meglinski, “Application of gold nanoparticles as contrast agents in confocal laser scanning microscopy”, Saratov Fall Meeting (SFM 2008), 23-26 September 2008, Saratov, Russia
- 4) **B. Veksler**, E. Kobzev, M. Bonesi, I. Meglinski, “Application of optical coherence tomography for imaging of scaffold structure and micro-flows characterization”, 1st Canterbury Workshop on Optical Coherence Tomography and Adaptive Optics (WSOCTAO), 8-10 September 2008, Canterbury, UK
- 5) **B. Veksler**, M. Kirillin, V. Kuzmin, I. Meglinski, “Simulation of Polarization-Sensitive Optical Coherence Tomography images by Monte Carlo method”, 11th Electromagnetic and Light Scattering Conference (ELS-XI), 7-12 September 2008, Hatfield, UK

- 6) G. Akchurin, G. Akchurin Jr., **B. Veksler**, I. Kolbenev, I. Maksimova, V. Maksimov, I. Meglinski, O. Naumova, A. Skaptsov, B. Khlebtsov, N. Khlebtsov, “Detecting of plasmon-resonate nanoparticles with low-coherent and confocal optical tomography for the purposes of laser phototermolysis”, V Meeting of Russian Society for Photobiology, 8-13 June 2008, Pushchino, Russia
- 7) **B. Veksler**, I. Meglinski, S. Alexandrov and D. Sampson, “Impact of probe geometry on the detecting field of view”, Saratov Fall Meeting (SFM 2007), 25-28 September 2007, Saratov, Russia.
- 8) **B. A. Veksler**, D. Y. Churmakov, V. L. Kuzmin, A. Y. Valkov, I. V. Meglinski, “New computational model for simulation of light propagation within biological tissues”, International Conference on Laser Applications in Life Sciences (LALS 2007), 11-14 June 2007, Moscow, Russia.
- 9) **B. A. Veksler**, A. V. Kovaleva, I. L. Maksimova, I. V. Meglinski, “Image reconstruction of tumour in tissue using method of artificial neural network”, International Conference on Laser Applications in Life Sciences (LALS 2007), 11-14 June 2007, Moscow, Russia.
- 10) **B. A. Veksler**, D. Y. Churmakov, V. L. Kuzmin, A. Y. Valkov, I. V. Meglinski, “New computational model for simulation of light propagation within biological tissues”, Third International Conference on Optical and Laser Diagnostics (ICOLAD 2007), 22-25 May 2007, London, UK.

Chapter 1. Introduction

Optical diagnostic techniques nowadays employed in different scientific areas of research from material science and research of space to biomedical applications (see, for example, Tuchin, 2002). Optical diagnostics in case of biomedicine can provide powerful tools for clinical studies in range from single molecules of organisms to biotissues and systems of organs. Currently the most used methods of optical diagnostics (see Figure 1.1) include X-ray radiography, which became feasible due to the discovery of X-rays in 1895 by Wilhelm Conrad Röntgen, ultrasound imaging (ultrasonography), introduced after World War II, X-ray computed tomography (CT), which was developed in 1970s and initiated digital cross-sectional imaging (tomography), and Magnetic Resonance Imaging (MRI), which was invented also in 1970s and enabled functional imaging with high spatial resolution. The main disadvantage of the techniques presented above is their danger influence on the human body, which can be avoided by using of the optical methods of diagnostics.

The recent rapid progress in non-invasive and safe imaging of micro- and/or macro-inhomogeneities inside various composite materials and biotissues has become feasible since the development of laser in 1960 (Maiman, 1960) with complimentary experimental tools, including optical fibres, Charge Coupled Device (CCD), synchroscan streak cameras, photomultiplier tubes, and photodiode detectors (Mignani and Baldini, 1996; Tuchin, 2002; Vo-Dinh, 2003; Verdaasdonk and van Swol, 1997; Udupa and Gabor, 1999; Splinter and Hooper, 2006). Number of new original optical based techniques were introduced in the past, basing on the usage of the diode lasers, broadband femtosecond lasers (Ti:Sapphire or Cr:Försterite) and superluminescence diodes (SLD) (Unterhuber *et al.*, 2004; Tuchin, 2004; Wang and Wu, 2007).

The important diagnostic tools for the non-invasive monitoring of diabetes (Jaap and Tooke, 1995), port wine stains studies (Kelly *et al.*, 2005), for surgery (Neligan, 1993) and for burn depth assessment (Niazi *et al.*, 1993), for diagnostics of atherosclerosis (Regar *et al.*, 2007) and for investigation mechanisms of photodynamic therapy at cancer treatments (Korbelik and Kroszl, 1994) are diffusing wave spectroscopy (DWS) (Harden and Viasnoff,

2001; Meglinski, 2003), which is an extension of the conventional Dynamic Light Scattering (DLS) (Cummins and Pike, 1973).

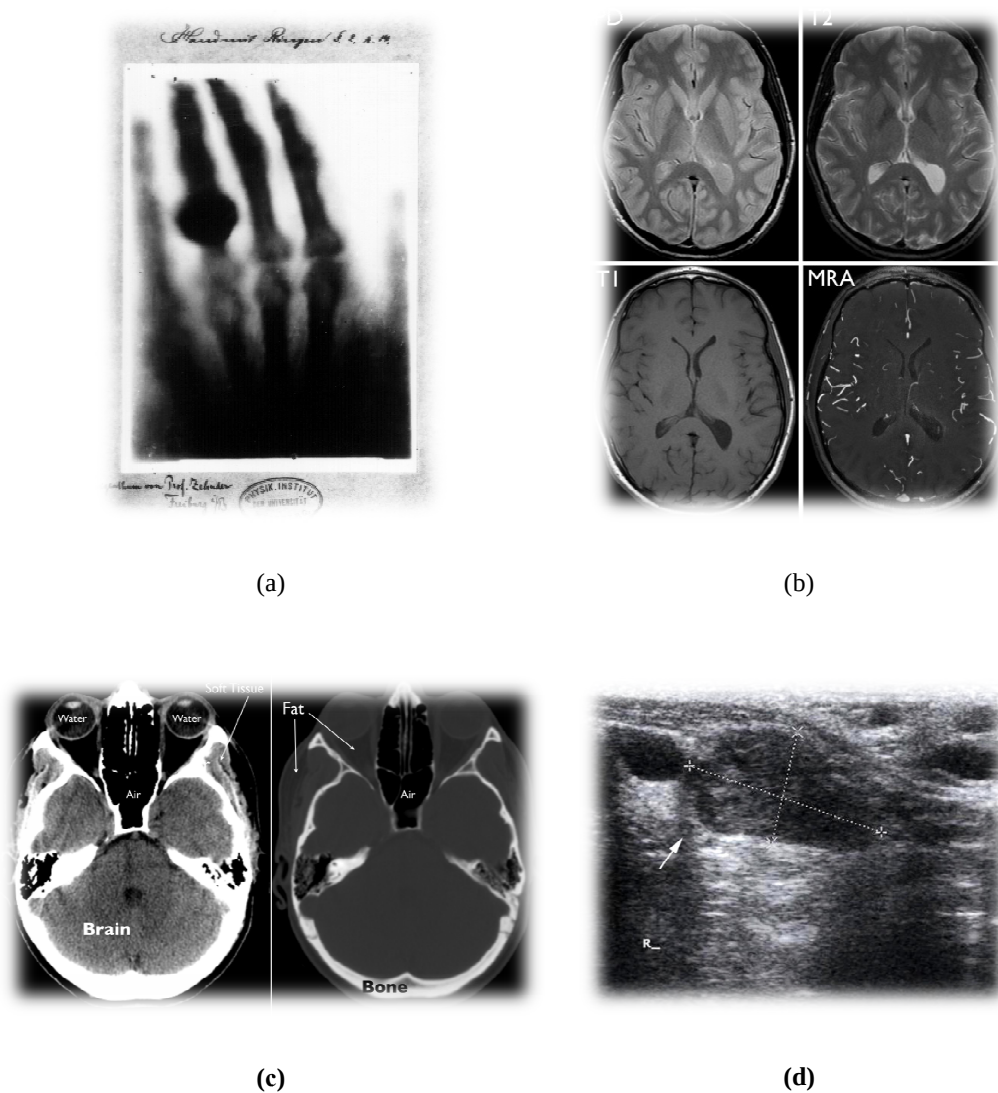


Figure 1.1. (a) - Wilhelm Röntgen's first "medical" X-ray of his wife's hand (Kevles B. H., 1996); (b) – MRI images of human brain (adopted from <http://knol.google.com/k/christopher-hess/brain-ct-mri>); (c) – CT images of human brain (adopted from <http://knol.google.com/k/christopher-hess/brain-ct-mri>); (d) - ultrasonography of the parathyroid glands (Selvi F. et al., 2009).

Another hot topic application of optical diagnostics is laser speckle imaging which can monitor dynamically cerebral blood flow (CBF) with high-resolution (Dunn *et al.*, 2001; Haiying *et al.*, 2004). By illuminating the cortex with laser light and imaging the resulting speckle pattern, relative CBF images with tens of microns spatial and millisecond temporal resolution were obtained (Luo *et al.*, 2004). Laser interferometry of random phase (light scattering) methodology is used for describing of spatially-modulated laser beam transportation through thin tissue layers or turbid thin films. This technique is applied for random object inspection (Angelsky and Maksimyak, 1990; Angelsky *et al.*, 1999), for surface roughness measurements (Taganov and Toporets, 1976; Ohlidal *et al.*, 1995), and for retinal visual acuity determination at cataract (Saleh, 1982; Enoch *et al.*, 1995; Radchenko *et al.*, 1999) and monitoring of scattering properties of blood during sedimentation and aggregation of erythrocytes (Gandjbakhche *et al.*, 1994; Tuchin *et al.*, 2002).

Optical heterodyne detection characterized by phase-sensitive measurements of light fields with a high signal-to-noise ratio (SNR) and large dynamic range is a relatively new and fast growing coherent-domain optical method. The technique, in its basic principles, is known as Optical Coherence Tomography (OCT) (Huang *et al.*, 1991; Schmitt, 1999; Fercher *et al.*, 2003; Drexler, 2004) and has found important applications in medicine, biology and material studies (Bouma and Tearney, 2002; Tuchin, 2004; Brezinski, 2006; Regar *et al.*, 2007). Optical Coherence Tomography is a fundamentally new type of optical imaging modality. OCT performs high resolution (Brezinski and Fujimoto, 1999; Drexler, 2004), cross-sectional tomographic imaging of the internal microstructure in materials (Chinn and Swanson, 2002; Dunkers, 2002) and biological systems (Fujimoto *et al.*, 1995) by measuring backscattered or backreflected light. Image resolutions of 1-15 μm can be achieved, which is one to two orders of magnitude higher than with conventional ultrasound (Brezinski *et al.*, 1997). Imaging can be performed non-invasively or *in situ* and in real time (several frames per second for 200-500 pixel images) (Bouma and Tearney, 2002; Brezinski, 2006). The unique features of this technology enable a broad range of

research and clinical applications. OCT can be interfaced with a wide range of imaging delivery systems and imaging probes (Li *et al.*, 2000; Hwang *et al.*, 2005). OCT technology is based on fiber optics and uses components developed for the telecommunications industry. Image information is generated in electronic form, which is amenable to image processing, and analysis, as well as electronic transmission, storage and retrieval. OCT systems can be engineered to be compact and low cost, suitable for applications in research, manufacturing or the clinic.

Most OCT systems have employed a reference delay scanning interferometer, such as a Michelson interferometer (Born and Wolf, 1986), in combination with a low coherence light source. There are many different embodiments of the interferometer and imaging engine for specific applications such as polarization diversity (insensitive) imaging, polarization-sensitive imaging and Doppler flow imaging. Polarization-sensitive detection techniques have been demonstrated using a dual channel interferometer (Hee *et al.*, 1992; de Boer *et al.*, 1997; Everett *et al.*, 1998). These techniques permit imaging of the birefringence properties of structures. Collagen and other tissues are strongly birefringent, and polarization-sensitive OCT (PS-OCT) can be a sensitive indicator of the tissue microstructural organization.

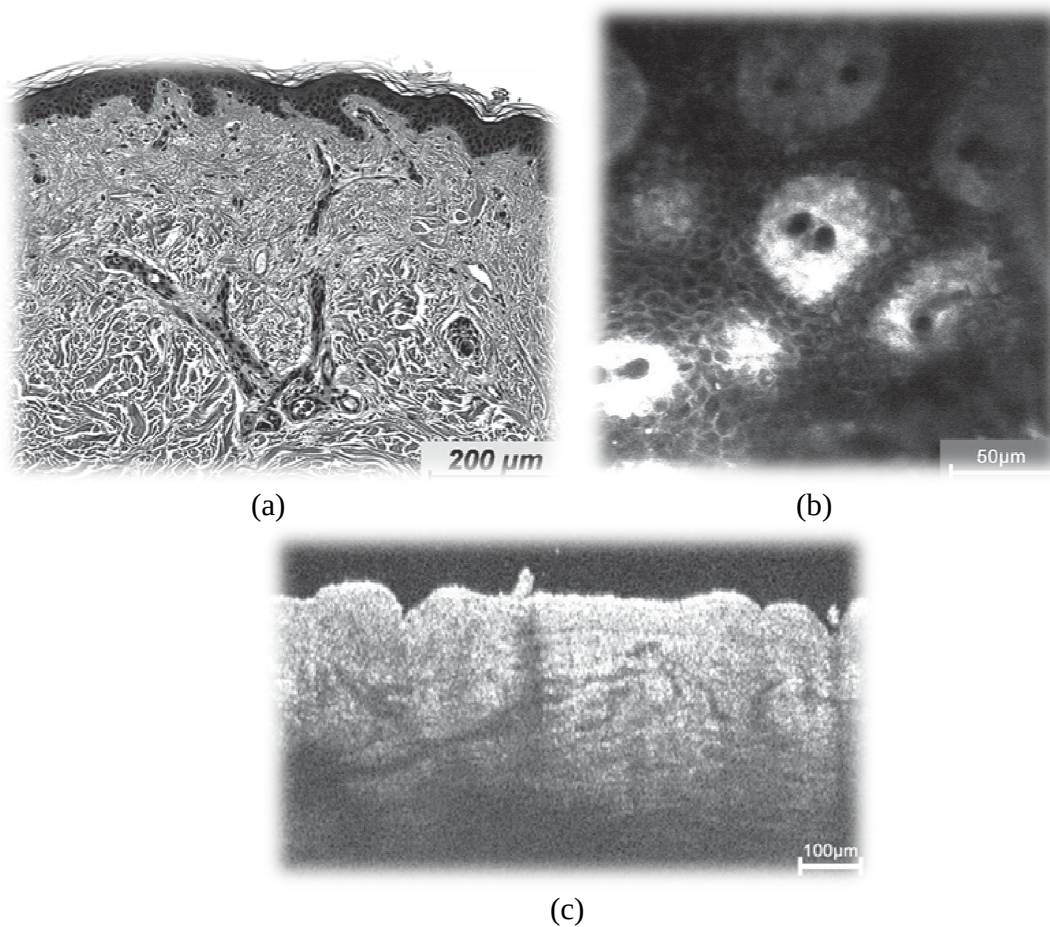


Figure 1.2. Internal structure of human skin with blood vessels. (a) – traditional histological representation; (b) – CLSM image of the same region; (c) – representation of the same area with OCT. (Lademann et al., 2008).

Besides the specific tools for optical diagnostics the invention of laser improved area of investigations with conventional microscopes. The confocal laser scanning microscopy (CLSM) (Sheppard *et al.*, 1997) offers several advantages over conventional fluorescence-based microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane, and the capability to collect serial optical sections from thick specimens (Sheppard *et al.*, 1997). CLSM nowadays has become an essential tool in biology and associated biomedical sciences, as well as in

materials science due to attributes that are not readily available using other contrast modes with traditional optical microscopy (Pawley, 1995; Paddock, 1999; Diaspro, 2002; Matsumoto, 2002; Sheppard and Shotton, 1997; Muller, 2002; Hibbs, 2004; Conn, 1999; Corle and Kino, 1996; Wilson, 1990; Gu, 1996; Masters, 1996). The application of a wide array of new synthetic and naturally occurring fluorochromes has made it possible to identify cells and sub-microscopic cellular components with a high degree of specificity amid non-fluorescing material (Mason, 1999). Comparison of CLSM, OCT and conventional histology is presented on the Figure 1.2, where the internal morphological structure of human skin is shown.

Flow imaging based on Doppler Optical Coherence Tomography (Doppler OCT) (Izatt *et al.*, 1996; Izatt *et al.*, 1997; Yazdanfar *et al.*, 1997; Chen *et al.*, 1997a; Chen *et al.*, 1997b; Chen *et al.*, 1998; Barton *et al.*, 1998; Yazdanafar *et al.*, 1998; Yazdanafar *et al.*, 2000) is an emerging technique and is still under development. The great advantage of this technique is its very high spatial resolution (1-10 μm), which, however, limits the penetration depth to around 1 mm. Its application *in vitro* experiments has shown the possibility to measure parabolic and non parabolic velocity profiles (Bonesi *et al.*, 2006; Bonesi *et al.*, 2007; Veksler *et al.*,) and distribution of red blood cells (RBCs) concentrations (Moger *et al.*, 2004). From these velocity profiles the local shear rate can be derived, as has been shown by van Leeuwen and co-workers (van Leeuwen *et al.*, 1999). *In vivo* applications were described in subsurface vessels of laboratory animals (Barton *et al.*, 1999; Rollins *et al.*, 2002; Wong *et al.*, 2002; Chang and Hou, 2003; Satomura *et al.*, 2004). Doppler OCT in human vessels concerned blood flow measurements in sub-superficial skin vessels, which allowed more advanced diagnosis and therapeutic control of cutaneous disorders (Tearney *et al.*, 2003; Pierce *et al.*, 2004; Gambichler *et al.*, 2005). Furthermore, OCT allows the assessment of the retinal blood flow dynamics and seems a promising technique for diagnosis of diabetic retinopathy and glaucoma (Yazdanafar *et al.*, 2003). Although the technique is still in development, including needle-based Doppler OCT (Yang *et al.*, 2005) and real-time flow assessment (Rollins *et al.*, 2002; Schaefer *et al.*, 2004; Leitgeb *et al.*, 2004), OCT application for coronary flow measurements has not

yet been shown. However, the use of OCT for research on the flow-related changes in atherosclerotic plaque is promising, because of its demonstrated capacity to differentiate plaque constituents *ex vivo* and *in vivo* (Regar *et al.*, 2003; Tearney *et al.*, 2003; Patel *et al.*, 2005; van der Meer *et al.*, 2005a; van der Meer *et al.*, 2005b; Regar *et al.*, 2007).

In current research the Monte Carlo (MC) technique was applied for the purposes of simulation of OCT. The MC simulation makes it possible to give a realistic picture of the processes involved into the light-tissue interaction. Although the modelling of optical propagation within biotissues with the MC is relatively time consuming, it is the most efficient technique to predict and analyze the peculiarities of the radiation propagation in the complex turbid medium. Existing MC code was added with coherence and polarization effects, which significantly increasing the accuracy of the modelling. MC model was used also for the purpose of visualization of the inhomogeneity in turbid medium with the help of artificial neural network (Kovaleva *et al.*, 2007). Low coherence optical techniques, such as optical coherence tomography (OCT), its extension Doppler OCT and confocal laser microscopy were improved and applied for the purposes of bio-tissues diagnostics and imaging of their internal structure. In addition CLSM was employed for the investigations of image contrasting with gold nanoparticles (Lemelle *et al.*, 2009).

Advantage of these techniques, especially OCT and Doppler OCT, is confirming with relatively low cost of building such instruments in comparison with cost of traditional diagnostic tools such as MRI or CT which is higher than \$100k. For example, the cost of the set-up used in the current research is lower than \$10k; however, it can be successfully used for different scientific purposes including flow measurements (Veksler *et al.*, 2008) and the research of gold nanoparticles. Hence, from the financial point of view return on invested money will be much higher in case of OCT and Doppler OCT, which make them more attractive for the usage in commercial projects.

Current PhD thesis consists with two big logical parts – theoretical and experimental. Further, in the second chapter the basic principles of light-tissue interaction are represented. Third one is describing the radiative transfer theory. In the fourth chapter the Monte Carlo

method as a method of solution of radiative transfer equation is described. Low-coherence techniques (OCT, Doppler OCT and CLSM) are reviewed in the fifth chapter. Last two chapters include experimental results obtained during my PhD studies in Cranfield. Sixth chapter contains the results of application and improvement of Monte Carlo simulations. And seventh chapter is showing the results of application of the low-coherence techniques and feasibility of their improvement.

Chapter 2. Light-tissue interaction

2.1 Electromagnetic wave theory of light

The life on the Earth owes the influence of Sun light its appearance. Light interaction with biological matter lies in the basis of function of different kinds of organisms. Furthermore such phenomenon allows us to reveal information about the morphological structure of biological tissues and understand processes within them.

So, let's consider what is 'light', what is its nature, main characteristics and how light can interact with the tissues.

The term 'light' refers to the quite small part of the electromagnetic spectrum between 400 nm and 700 nm, so called visible light (Figure 2.1) (Born and Wolf, 1986).

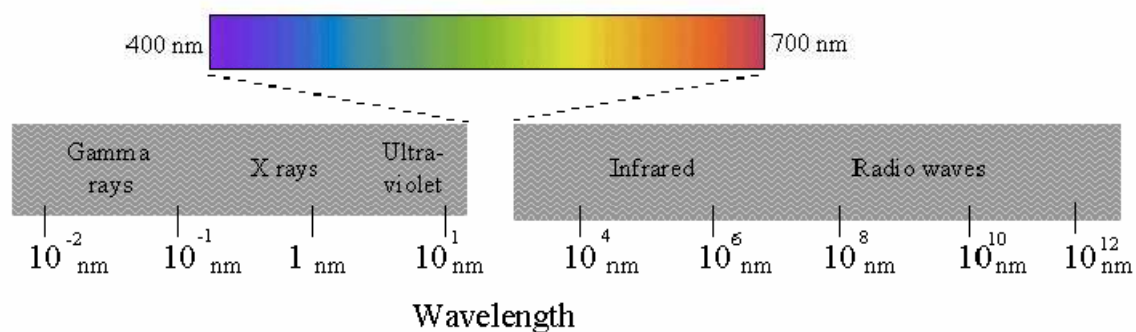


Figure 2.1. Electromagnetic radiation spectrum, representing optical range 400-700 nm.

The physical nature of light is dual, so it can be considered as both particles and waves at the same time. Light as electromagnetic wave has different characteristics, such as amplitude, wavelength and polarization. The behaviour of electromagnetic waves has been described by the Maxwell Equations until Planck, Einstein and others developed the quantum theory, considering the light in terms of photons. From this point of view the

brightness of the light is the number of photons, and the colour of the light is the energy contained in each photon (Born and Wolf, 1986).

Wave nature of light can be described with the next basic parameters: wavelength λ , or frequency ν . Whereas, the particle description of the radiation requires using the energy characteristics E . As soon as both types of light representation must be equal, it is possible to write the next equation:

$$E(J) = h\nu = h\frac{c}{\lambda} = E(eV)q_e, \quad (2.1)$$

where h is the Planck's constant, c is the speed of light, and q_e is the the electron unit charge. $E(J)$ and $E(eV)$ are energy in joules and energy in electron-volts; respectively.

Biological tissues can react on the electromagnetic radiation in different ways. The human body is more or less transparent for the low frequency radio waves and for the X-rays range. And in the region of microwaves, infrared and UV ranges the human tissues absorb optical radiation.

Depending on the conditions it is possible to take into account both types of description of light nature in tissue optics. For example, while studying tissues with regular structure such as flowing blood containing disc-shaped red-blood cells the wave properties of radiation can be considered. Whereas, in the case of thick and dense tissues, such as breast, a model based on the light propagation as a stream of energy quanta takes an advantage.

For the first time the phenomena of electromagnetic waves was described in the form of equations by James Clerk Maxwell in 1865, which are known nowadays as Maxwell equations. Maxwell noticed in his equations that changes in the electric field determine changes in the magnetic field, and vice versa. Thus, if we are describing light as an electromagnetic wave it will satisfy Maxwell's theory. Below these equations are represented in SI units (Born and Wolf, 1986):

$$\nabla \mathbf{D} = \rho_F, \quad (2.2)$$

$$\nabla \times \mathbf{E} + \frac{\partial \mathbf{B}}{\partial t} = 0, \quad (2.3)$$

$$\nabla \mathbf{B} = 0, \quad (2.4)$$

$$\nabla \times \mathbf{H} = \mathbf{J}_f + \frac{\partial \mathbf{D}}{\partial t}, \quad (2.5)$$

where $\mathbf{E}$ is the electric field, $\mathbf{B}$ is the magnetic induction, $\mathbf{J}_f$ is the current density, and ρ is the volume charge density. $\mathbf{D}$ and $\mathbf{H}$ are defined by (Born and Wolf, 1986)

$$\mathbf{D} = \varepsilon_0 \mathbf{E} + \mathbf{P}, \quad (2.6)$$

$$\mathbf{H} = \frac{\mathbf{B}}{\mu_0} - M, \quad (2.7)$$

where $\mathbf{D}$ is the displacement, $\mathbf{H}$ represents the magnetic field, $\mathbf{P}$ is the electric polarization, and M is the magnetization. The permittivity and the permeability of the vacuum are represented by ε_0 and μ_0 , respectively. All the Eqs. (2.2) to (2.7) must be supplemented with the following constitutive relations (Born and Wolf, 1986)

$$\mathbf{J} = \sigma \mathbf{E}, \quad (2.8)$$

$$\mathbf{B} = \mu \mathbf{H}, \quad (2.9)$$

$$\mathbf{P} = \varepsilon_0 \chi \mathbf{E}, \quad (2.10)$$

where σ is the conductivity, μ is the permeability, and χ is the electric susceptibility.

These coefficients are the individual characteristics of the considered medium. In case of non-scattering medium the latter can be approximated by homogeneous and isotropic set of dipoles (Born and Wolf, 1986). So, σ , μ and χ can be assumed as independent to position and direction (Born and Wolf, 1986).

The Maxwell equations may be written in complex form as (Born and Wolf, 1986)

$$\nabla(\varepsilon_r \varepsilon_0 \mathbf{E}(r, \omega)) = 0, \quad (2.11)$$

$$\nabla \times \mathbf{E}(r, \omega) = i\omega\mu_0 \mathbf{H}(r, \omega), \quad (2.12)$$

$$\nabla \mathbf{H}(r, \omega) = 0, \quad (2.13)$$

$$\nabla \times \mathbf{H}(r, \omega) = -i\omega\varepsilon_r \varepsilon_0 \mathbf{E}(r, \omega), \quad (2.14)$$

where r is the position vector, ε_r is the complex permittivity, and ω is the angular frequency of the harmonic fields. From Eq. (2.11) to (2.14), the complex permittivity is given by

$$\varepsilon_r = \varepsilon_0(1 + \chi(\omega)) + i \frac{\sigma}{\omega}, \quad (2.15)$$

Propagation of light is describing with the help of the wave equations, which are based on the Maxwell equations and presented below (Born and Wolf, 1986; Ishimaru, 1978):

$$\nabla^2 \mathbf{E}(r, \omega) + \frac{\omega^2 n_c^2}{c_0^2} \mathbf{E}(r, \omega) = 0, \quad (2.16)$$

$$\nabla^2 \mathbf{H}(r, \omega) + \frac{\omega^2 n_c^2}{c_0^2} \mathbf{H}(r, \omega) = 0, \quad (2.17)$$

where c_0 is the speed of light in vacuum, and n_c is the complex refractive index that is defined by

$$n_c = n + i\kappa = \sqrt{\varepsilon}. \quad (2.18)$$

The solutions of the Eq. (2.16) and (2.17) are:

$$\mathbf{E} = \mathbf{E}_0 \exp(i\mathbf{k}x - i\omega t), \quad (2.19)$$

$$\mathbf{H} = \mathbf{H}_0 \exp(i\mathbf{k}x - i\omega t), \quad (2.20)$$

where $\mathbf{E}_0$ and $\mathbf{H}_0$ are constant vectors, and $\mathbf{k}$ is the wave vector indicating the direction of propagation of light. The wave vector may be complex (Born and Wolf, 1986; Ishimaru, 1978):

$$\mathbf{k} = \mathbf{k}' + i\mathbf{k}'', \quad (2.21)$$

where $\mathbf{k}'$ and $\mathbf{k}''$ are real vectors.

Substituting Eq. (2.21) in Eqs. (2.19) and (2.20), it is possible to obtain

$$\mathbf{E} = \mathbf{E}_0 \exp(-\mathbf{k}''x) \exp(i\mathbf{k}'x - i\omega t), \quad (2.22)$$

$$\mathbf{H} = \mathbf{H}_0 \exp(-\mathbf{k}''x) \exp(i\mathbf{k}'x - i\omega t), \quad (2.23)$$

$\mathbf{E}_0 \exp(-\mathbf{k}''x)$ and $\mathbf{H}_0 \exp(-\mathbf{k}''x)$ are the amplitudes of the electric and magnetic waves, and $(i\mathbf{k}'x - i\omega t)$ is the phase of the waves (Born and Wolf, 1986). If $\mathbf{k}'$ and $\mathbf{k}''$ are parallel, the waves are said to be homogeneous; if $\mathbf{k}'$ and $\mathbf{k}''$ are not parallel, the waves are said to be inhomogeneous (such as waves propagating in vacuum) (Born and Wolf, 1986; Ishimaru, 1978).

Parts of electromagnetic field ($\mathbf{E}$, $\mathbf{H}$), its electrical and magnetic compounds, are connected together with the next quantity, so called Poynting vector (Born and Wolf, 1986):

$$\mathbf{S} = \mathbf{E} \times \mathbf{H}. \quad (2.24)$$

Poynting vector is a rapidly varying function of frequency for the time-harmonic fields, thus, usually the time-averaged form of vector $\mathbf{S}$ is used:

$$\langle \mathbf{S} \rangle = \frac{1}{2} \text{Re} \{ \mathbf{E} \times \mathbf{H}^* \}. \quad (2.25)$$

For the case of a plane harmonic wave it is possible to represent the time-averaged Poynting vector as (Born and Wolf, 1986):

$$\langle \mathbf{S} \rangle = \frac{1}{2} n c_0 \varepsilon_0 |\mathbf{E}_0|^2 e^{\frac{4\pi k z}{\lambda_0}} \mathbf{e}_z. \quad (2.26)$$

The magnitude of the time-averaged Poynting vector, which is denoted by the symbol I , is called irradiance,

$$I = |\langle \mathbf{S} \rangle|. \quad (2.27)$$

The dimension of irradiance is energy per unit area and time; and so often, the term intensity is used to denote irradiance.

From Eq. (2.26) it is not only seen that irradiance is in the direction of propagation, i.e. $\mathbf{e}_z$; and therefore parallel to $\mathbf{k}$; but also it appears that as the wave transverses the medium, the irradiance is also exponentially attenuated as a function of distance in the propagation direction (Born and Wolf, 1986).

2.2 Effects of interaction between radiation and medium

2.2.1 Absorption

It is possible to mention from Eq. (2.26) that intensity during the propagation of light within the medium is decreasing its value exponentially (Figure 2.2). Such dependence bears the name of Beer-Lamberts law (Tuchin, 2002; Tuchin, 2004):

$$I = I_0 e^{-\mu_a z}, \quad (2.28)$$

where I_0 is the intensity at $z = 0$, and μ_a is the absorption coefficient.

$$\mu_a = \frac{4\pi k}{\lambda_0}. \quad (2.29)$$

Therefore, it is clearly seen from the Beer-Lamberts law, that the absorption coefficient μ_a is directly proportional to the imaginary part $\mathbf{k}$ of the complex refractive index n_c , where $n_c = n + i\mathbf{k}$. Here n is the refractive index indicating the phase velocity of radiation of a

specific frequency in a specific material. This definition is based on the macroscopic electromagnetic term with a continuum medium assumption (Born and Wolf, 1986; Bohren and Huffman, 1983).

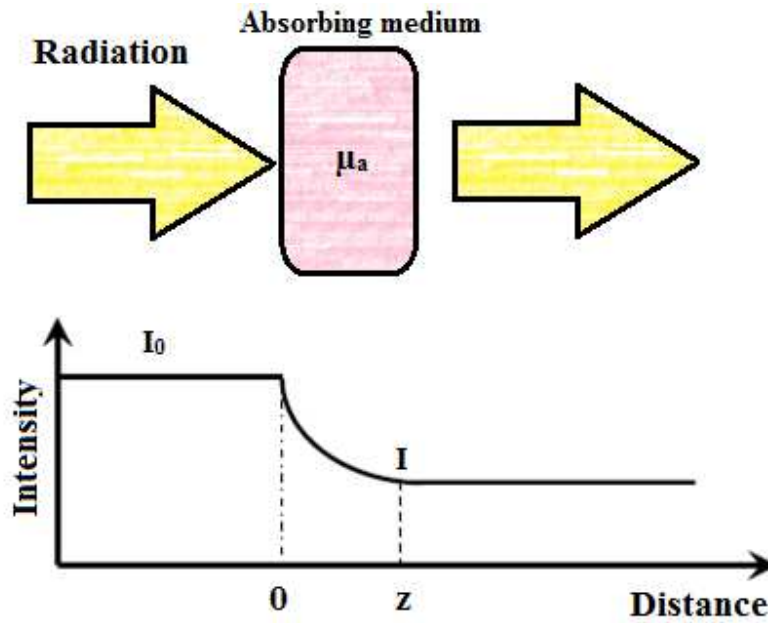


Figure 2.2. Schematic representation of exponentially decreasing of light intensity due to the absorption; z is the thickness of the sample, I_0 is the intensity of incident radiation at $z = 0$, I is the intensity of signal and μ_a is the absorption coefficient.

The absorption coefficient μ_a is showing the probability of photons absorption in the medium. Beer-Lamberts law can be used for the quantitative measuring of the absorption in cases of homogeneous and non-scattering media. At the same time, in the turbid medium, such as breast tissue, the path length of photons is not obvious and it is impossible to use Beer-Lambert law directly.

2.2.1.1 Aspects of absorption in the context of quantum theory

Understanding of the process of absorption of light within the medium is impossible without detailed description of the energetic changes in the medium during the light propagation. According to the quantum theory of absorption (Dirac, 1927) the energy levels in atoms and molecules are quantized. This fact is approving with the microscopic point of view. The absorption can be possible if the energy of the incident photon ($h\nu$) is high enough to create transitions between the energy levels in the molecule, otherwise this exact medium will be transparent for the radiation with the current wavelength.

There are two types of transitions between energy levels (Born and Wolf, 1986):

- high energy electronic transitions (UV and VIS regions);
- low energy vibrational or rotational transitions (NIR and IR regions)

On the Figure 2.3 the specific diagram representing these phenomena is shown (so called Jablonski diagram). Absorption of the photon causes excitation to the higher energy level $S_0 \rightarrow S_n$. Subsequently the molecule relaxes down to the lowest excited energy level. Depending on the different conditions there are few types of relaxation from the lowest excited state to the ground state. The relaxation is called fluorescence if the spin state of the initial and the final energy levels is the same ($S_1 \rightarrow S_0$). Otherwise ($T_1 \rightarrow S_0$), the relaxation is called phosphorescence.

Fluorescence and phosphorescence are radiative processes. Transitions between the same spin states are called internal conversion (IC), while such transitions between different spin states are called intersystem crossing (ISC) (Born and Wolf, 1986). Usually the stable ground state in most molecules is the singlet state (S_0 in Figure 2.3), while the triplet states ($T_{1,2}$ in Figure 2.3) are unstable excited states with an increased internal energy. Oxygen is one of the rare molecules for which the lowest triplet state is the ground state. Excited molecular oxygen is in the singlet state. This is important in the photodynamical reaction occurring, for example, during photodynamic therapy (PDT) (Dougherty *et. al.*, 1998).

There is another type of photon-electron transition called stimulated emission, which happens in atoms.

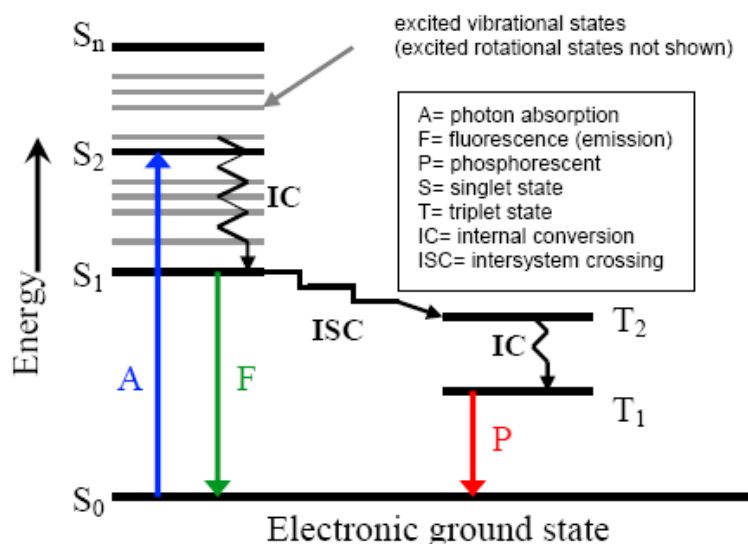


Figure 2.3. Jablonski diagram. Energy levels and the possible pathways following light absorption by an absorbing molecule (chromophore).

When an atom or molecule already is in an excited state, then an incoming photon with quantum energy equal to the excess internal energy of that atom or molecule, can produce a second photon of the same energy by stimulating the atom or molecule to fall down to its lower energy state, shown in Figure 2.4. This is the principle of the light amplification happens in lasers (Light Amplification by Stimulated Emission of Radiation). Photons produced by stimulated emission have the same phase and frequency, resulting in very intense and coherent light (laser).

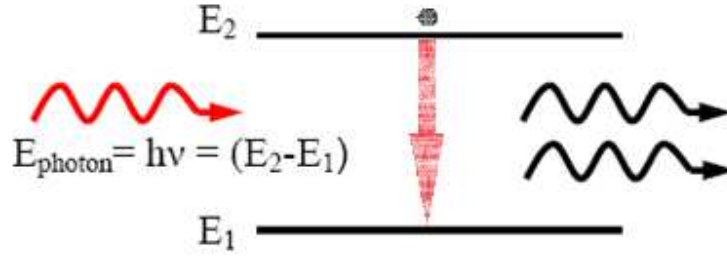


Figure 2.4. Stimulated emission. The electron in the excited state falls into the lower state emitting a photon. Here, E_1, E_2 is the atomic energy states, E_{photon} is the energy of emitted photon.

2.2.2 Absorption within biomedical tissues

Inside biological tissue, light can be absorbed by tissue absorbers, called chromophores. The probability of absorption inside bio-tissues is described by μ_a , which is defined as the probability of absorption per unit length. The absorption coefficient can be considered as the sum of all the contributions to absorption by chromophores in the tissue, as the following

$$\mu_a^\lambda = \ln(10) \varepsilon_x^\lambda C_x, \quad (2.30)$$

where ε_x^λ is the molar extinction coefficient, and C_x is the absorber concentration of the chromophore. The product of $\varepsilon_x^\lambda C_x$ is in units of cm^{-1} . From Eq. (2.30) it is seen that absorption coefficient is wavelength depended as a result of different chromophores have different absorption spectra. Figure 2.5, shows the absorption spectra of some important chromophores in human skin tissue.

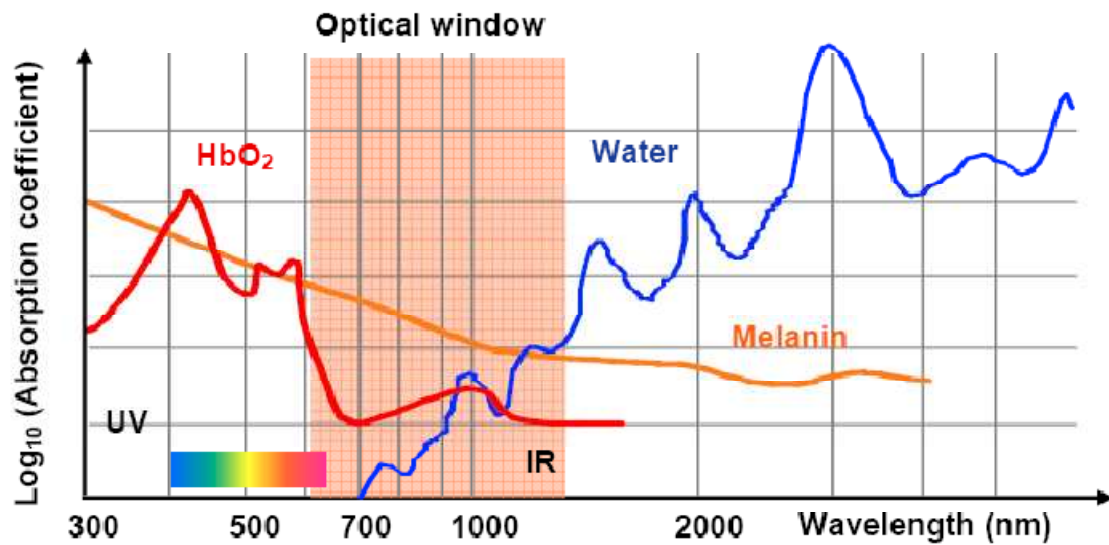


Figure 2.5. Absorption spectra of main chromophore presented in human skin tissue, as function of wavelength (Tuchin, 2002; Meglinski and Matcher, 2002).

Constituting 75 % of the tissue, water is the most important absorber in tissue, dominating in the UV ($\lambda < 200 \text{ nm}$), and IR ($\lambda > 1300 \text{ nm}$) region (Tuchin, 2002). Other important chromophores in tissue are haemoglobin and melanin. The absorption of light by blood depends on the oxygenated (HbO₂) and deoxygenated (Hb) haemoglobin. The absorption spectra shows one sharp peak at about 430 nm for HbO₂, while dropping off until 530 nm, the spectra picks up at 540 nm and 576 nm, and finally its lowest absorption takes place for wavelengths above 600 nm (Figure 2.5). Melanin is a pigment in hair, skin and eye. Protecting the organism from UV radiation is the main function of melanin in the skin. Melanin absorption decreases with increasing wavelength. The low-absorbing region between approximately 630 nm and 1300 nm is referred to as the tissue optical window. In this range, the penetration of light into biological tissue is at its deepest.

2.2.3 Refraction and reflection within the medium

Light incident on a surface of tissue, will be partially reflected and partially transmitted as a refracted ray. Due to interaction of light with the medium, the effective speed of light is reduced. This reduction is determined by the real part of the refraction index of the medium. The real part of the index of refraction is defined as the ratio between the speed of light in vacuum and the speed of light in the medium.

$$n = \frac{c}{v}. \quad (2.31)$$

The angle relationships for both reflected and refracted light can be derived according to Fermat's principle. Fermat's principle says that light follows the path of least time. Considering Figure 2.6, the following shows how the law of reflection can be derived from the Fermat's principle. The path length L , along the light ray from A to B is

$$L = \sqrt{a^2 + x^2} + \sqrt{b^2 + (d - x)^2}. \quad (2.32)$$

As the speed of light is constant, we can obtain the point of reflection yielding the minimum path distance by setting the derivative of L with respect to x equal to zero

$$\frac{x}{\sqrt{a^2 + x^2}} = \frac{(d - x)}{\sqrt{b^2 + (d - x)^2}}, \quad (2.33)$$

which results in

$$\sin \theta_i = \sin \theta_r \text{ yielding } \theta_i = \theta_r$$

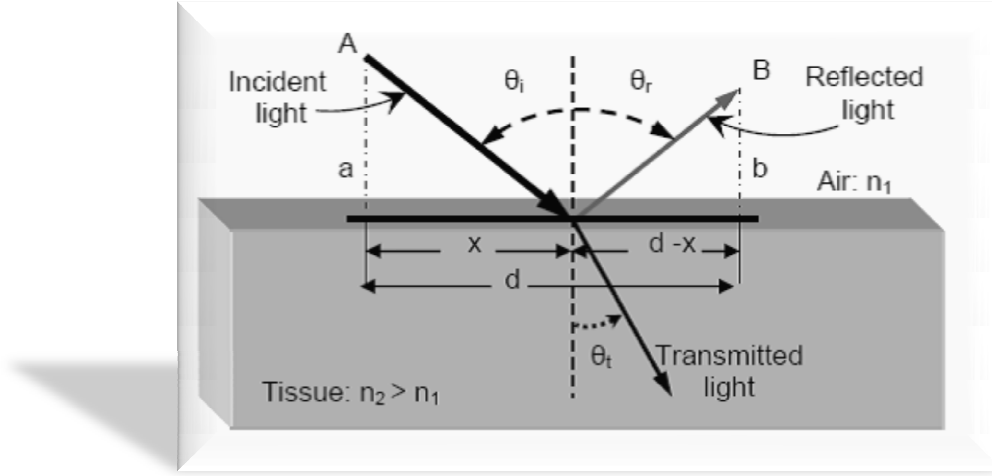


Figure 2.6. Schematic representation of light incident to the surface of a medium. Here, θ_i is the angle of incidence, θ_r is the angle of reflection, θ_t is the angle of refraction, n_1 and n_2 are refractive indexes in air and tissue respectively.

The above results show the law of reflection. According to the law of reflection, a light ray incident upon a reflective surface will be reflected at an angle equal to the incident angle with respect to the normal to the surface. As mentioned in the beginning of this section, the index of refraction is defined as the speed of light in vacuum divided by the speed of light in the medium, Eq. (2.31); so the index of refraction for different media can be measured. Snell's law relates the indices of refraction of the two media to the directions of propagation in terms of the angles to the normal. Considering Figure 2.7, it is seen that Snell's law can also be derived from Fermat's principle, by setting the derivative of time equal to zero ($n_1 < n_2$).

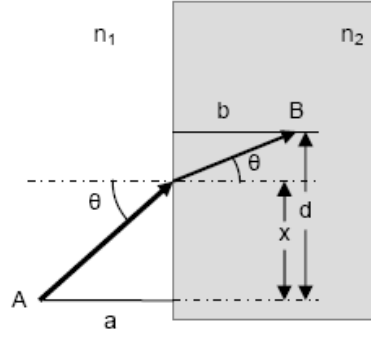


Figure 2.7. Schematic representation of light travelling from one medium (n_1) into another one (n_2) with $n_1 < n_2$, θ is the angle of incidence and refraction.

$$t = \frac{\sqrt{a^2 + x^2}}{v_1} + \frac{\sqrt{b^2 + (d - x)^2}}{v_2}, \quad (2.34)$$

$$\frac{dt}{dx} = \frac{x}{v_1 \sqrt{a^2 + x^2}} - \frac{(d - x)}{v_2 \sqrt{b^2 + (d - x)^2}}, \quad (2.35)$$

$$\frac{\sin \theta_1}{v_1} = \frac{\sin \theta_2}{v_2}. \quad (2.36)$$

If the index of refraction is defined as $n = \frac{c}{v}$ it is possible to represent Snell's law from (2.36) as

$$\frac{n_1}{n_2} = \frac{\sin \theta_2}{\sin \theta_1}. \quad (2.37)$$

In case of unpolarized light the specular reflectance R , which is the fraction of the reflected intensity, is given by Fresnel's formula (Born and Wolf, 1986),

$$R = \frac{1}{2} \left(\frac{\tan^2 (\theta_i - \theta_t)}{\tan^2 (\theta_i + \theta_t)} + \frac{\sin^2 (\theta_i - \theta_t)}{\sin^2 (\theta_i + \theta_t)} \right). \quad (2.38)$$

Using Snell's law it is possible to evaluate so called critical angle in case of light reflected in a surface of two media with different index of refraction $n_1 < n_2$. There will be no light refracted beyond the critical angle; however, all of the light will be reflected. For the case of internal reflection inside the sample the critical angle is presented as

$$\theta_c = \arcsin \left(\frac{n_1}{n_2} \right) \cong 46^\circ. \quad (2.39)$$

2.2.4 Scattering of light inside bio-tissues

In tissue optics, turbid biological media are considered as a quantity of discrete randomly distributed scattering particles (Figure 2.8). As the index of refraction of these discrete elements, n_s , is different from that of the surrounding medium, n_m ; the relative refractive index is introduced as the following,

$$m = \frac{n_s}{n_m} \neq 1. \quad (2.40)$$

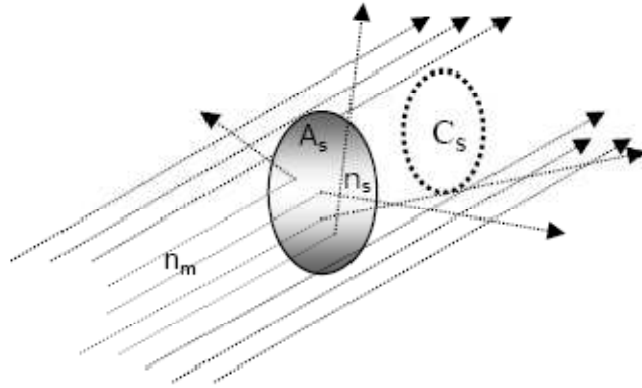


Figure 2.8. Process of scattering by a discrete particle; A_s is the scattering element, C_s is the effective scattering cross- section, n_s and n_m are the refraction indexes of the

Light passing through tissue can be attenuated both by absorption and by scattering. Only part of the light that is transported into the biological tissue will be transmitted. Light attenuation in tissue depends on wavelength of the incident light, and the tissue characteristics. Here, the scattering will be discussed. It should be emphasized here that there are two major types of scattering event. If the frequency of the scattered wave is equal to that of the incident wave, it is called elastic scattering, but if the frequency of the incident wave and the scattered wave differs, it is called inelastic scattering. Rayleigh and Mie scattering are two types of elastic scattering. Rayleigh scattering happens when the size of the scatterers (molecules and very tiny particles) are much smaller than the wavelength of the incident wave (up to about a tenth of the wavelength). Mie scattering predominates when the size of the scatterers are larger than the wavelength of the incident light. In Mie scattering the intensity of the scattered light increases with shorter wavelength, approximately proportional to λ^{-2} ; while in Rayleigh scattering, it is proportional to λ^{-4} . It is helpful to consider the molecular structure to understand the inelastic scattering event. A molecule is formed by the binding of two or more atoms in such a way that the total

energy is lower than the sum of the energies of the constituents. The description of molecular structure is considerably more complicated than that of isolated atoms since, apart from energy levels corresponding to different electronic arrangements, there are also different states corresponding to vibrational and rotational. The allowed internal energy for molecular structures of diatomic molecules is schematically shown in Figure 2.9.

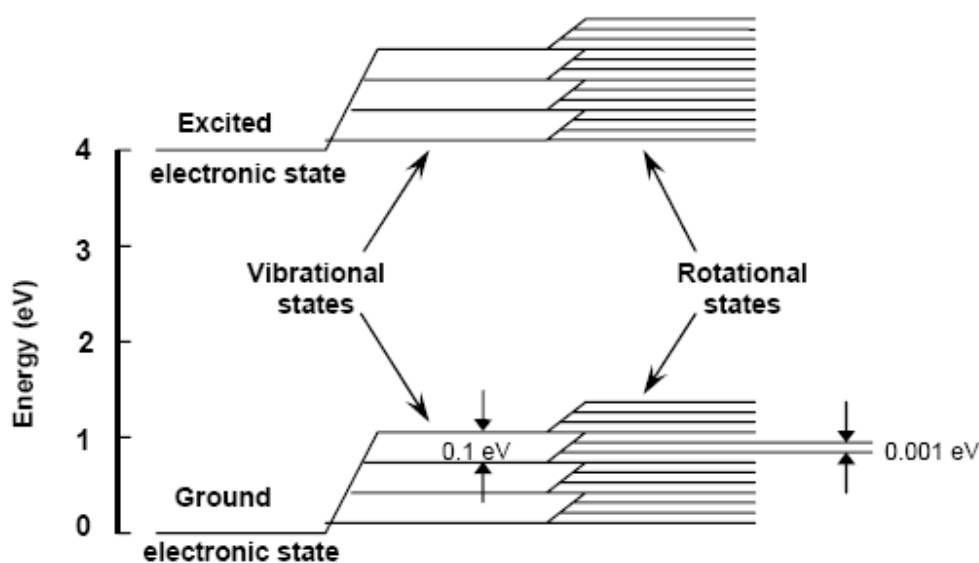


Figure 2.9. Schematic representation of the molecular energy levels for diatomic molecules.

Inelastic scattering happens when the incident light interacts with vibrational and rotational levels of molecules causing transition between these levels, and altering the wavelength (energy) of the scattered light comparing to the incoming light, see Figure 2.10. The Raman effect represents inelastic scattering. If the energy of the incident light is not corresponding to the energy separation between two electronic levels, still weak scattering effects, Rayleigh and Raman scattering, are obtained. As illustrated in Figure 2.10, depending on the initial and final states, energy of photon is preserved (Rayleigh scattering), increased (Raman Anti-Stokes) and decreased (Raman Stokes). In quantum mechanical theory, virtual levels are introduced, which mediate the scattering.

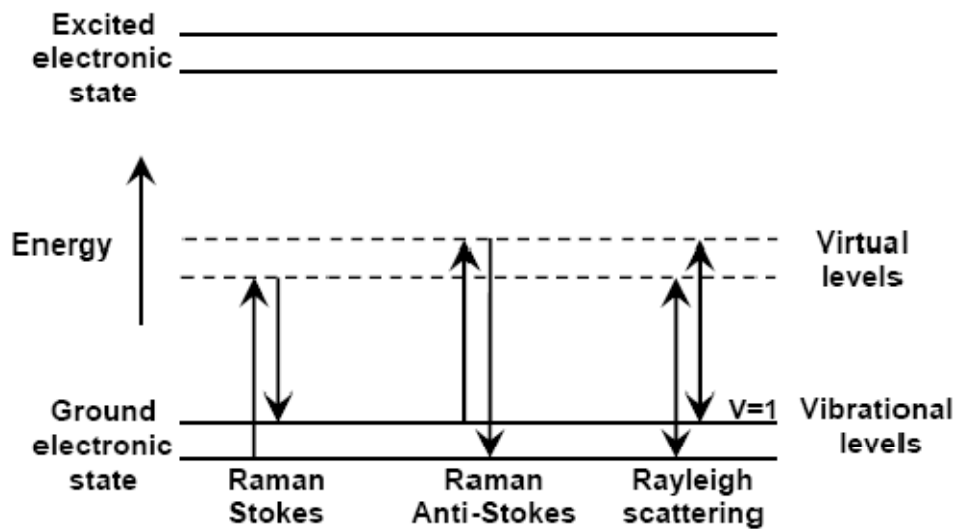


Figure 2.10. Schematic representation of energy transitions between molecular energy levels in cases of Raman and Rayleigh scattering.

Scattering in tissue is due to several optical effects; a) reflection and refraction of light from interfaces between materials having different refractive indices, b) reflection of light by discrete particles in the tissue ranging from organic molecules to whole cells, c) absorption of light rays by atoms and molecules and re-emission at the same wavelength but in other directions. In all these processes just the direction of light changes due to the scattering, and no energy loss is involved, so they are under the label elastic scattering. Scattering in turbid media is described by the scattering coefficient, μ_s , which is defined as the probability of scattering per unit length; and describes the average of how many times per unit length a photon change its direction. In tissue, scattering is not isotropic, but forward directed.

2.2.4.1 Scattering cross- section, coefficient, and mean free path length

Considering Figure 2.8, the effective scattering cross- section, C_s is defined as

$$C_s = \delta_s A_s, \quad (2.41)$$

where A_s is the scattering element through the scattering efficiency δ_s . Hence, the scattering coefficient is concluded as

$$\mu_s = C_s \rho_s, \quad (2.42)$$

where ρ_s is the volume density of the scattering elements. The average distance a photon travels between scattering events, is called the mean free path length, and is defined as

$$l = \frac{1}{\mu_s}. \quad (2.43)$$

2.2.4.2 Scattering phase function and anisotropy factor

In biological tissue the scattering of light is not isotropic, but has been shown to be strongly forward directed (Tuchin, 2002). Also, neither Rayleigh nor Mie scattering completely describe scattering in tissue. Therefore, it is very convenient to define a probability function $p(\theta)$ of a photon to be scattered by an angle θ , which can be fitted to experimental data. By furthermore assuming that tissue is isotropic in terms of physical properties (such as refractive index, density, etc.) scattering depends only on the angle θ between unit vector directions $\hat{s}$ and $\hat{s}'$ (Figure 2.11). The angular dependence of scattering, called the probability distribution function and/or the scattering phase function, is a function of the scattering angle such that $p(\hat{s}, \hat{s}') = p(\theta)$.

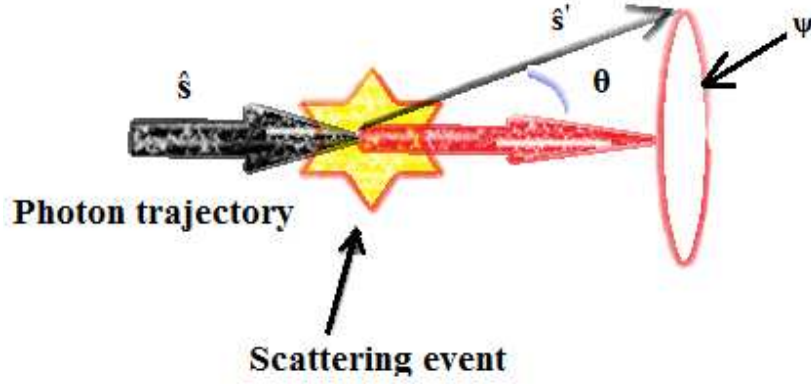


Figure 2.11. A scattering event causes a deflection at angle θ from the original direction $\hat{s}$ to the direction $\hat{s}'$, ψ is the azimuthal angle.

For light transport in biological tissue the Henyey-Greenstein function is usually applied as the following (Henyey and Greenstein, 1941):

$$p(\theta) = \frac{1}{4\pi} \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{3/2}}, \quad (2.44)$$

such that

$$\int_0^\pi p(\theta) 2\pi \sin\theta d\theta = 1, \text{ and } \int_0^\pi \cos\theta p(\theta) 2\pi \sin\theta d\theta = g.$$

It is also common practice to express the Henyey-Greenstein function as the function of $p(\cos\theta)$:

$$p(\cos\theta) = \frac{1}{2} \frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{3/2}}, \quad (2.45)$$

such that

$$\int_{-1}^1 p(\cos\theta) d(\cos\theta) = 1, \text{ and } \int_{-1}^1 \cos\theta p(\cos\theta) d(\cos\theta) = g,$$

where " g ", called anisotropy factor, is the mean cosine of scattering angle θ ($g = \cos\theta$), which measures the degree of anisotropy in scattering. Figure 2.12, shows a series of Henyey-Greenstein functions corresponding to different g - values.

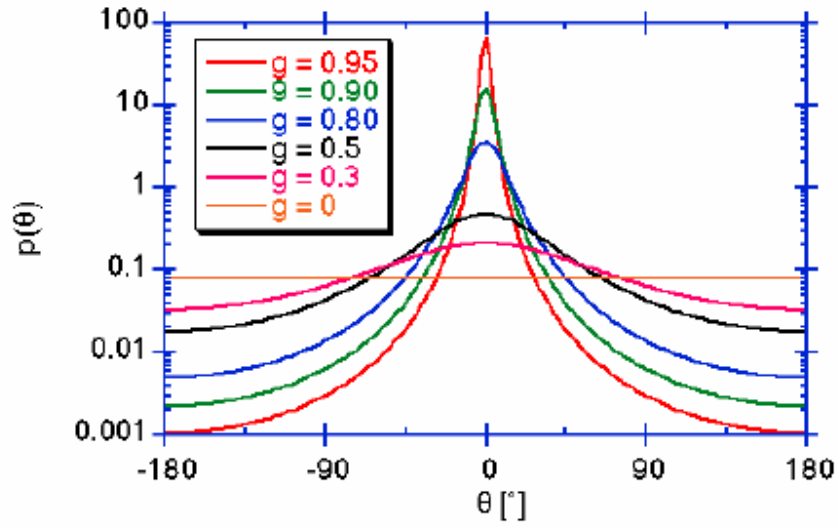


Figure 2.12. A series of plots of the Henyey-Greenstein functions for different g -values as a function of scattering angle (Ishimaru, 1978).

The g -factor ranges between -1 (for totally back scattering), and 1 (for totally forward scattering); while $g = 0$ corresponds to isotropic scattering. For most biological tissues the value of " g " varies between 0.8 and 0.9541. For *in vitro* tissues in the visible and near-infrared wavelength regions, " g " has been measured to be between 0.7 and 0.9938.

2.2.4.3 Mie scattering

Very often, the scattering medium behaves like a collection of discrete spherical particles. In this case the Mie scattering, which in its pure form deals with spherical particles, will dominate.

The Mie-scattering theory also called Lorenz-Mie, was developed by Gustav Mie in 1908. The theory is named after German physicist Gustav Mie (1868-1957) and Danish physicist Ludvig Lorenz (1829-1891), who independently developed the theory of electromagnetic plane wave scattering by a dielectric sphere. Mie-scattering theory provides rigorous solutions on the basis of electromagnetic theory for light scattering by an isotropic sphere embedded in a homogeneous medium. According to Mie theory, the scattering properties of non-absorbing homogeneous particle is described in terms of two parameters; the magnitude of refractive index mismatch, and the size of the surface of refractive index mismatch as the following,

$$x = 2\pi a(\lambda n_m), \quad (2.46)$$

where a is the size of sphere, λ is the wavelength of incident radiation and n_m is the refraction index of scattering medium.

In general, when the particle diameter is similar to the light wavelength, light interacts with the particle over an effective cross-sectional area larger than the geometrical cross section of the particle. The Mie calculation output provides this scattering cross section, C_s . Often this parameter is divided by the geometrical cross-sectional area to give a dimensionless scattering efficiency parameter:

$$\delta_s = \frac{C_s}{A_s}, \quad (2.47)$$

where δ_s is the efficiency of scattering and $A_s = a^2$ is the true geometrical crosssectional area of the particle. The scattering coefficient can be related to the scattering number density, ρ_s , and the cross-sectional area of scattering as,

$$\mu_s = \rho_s C_s. \quad (2.48)$$

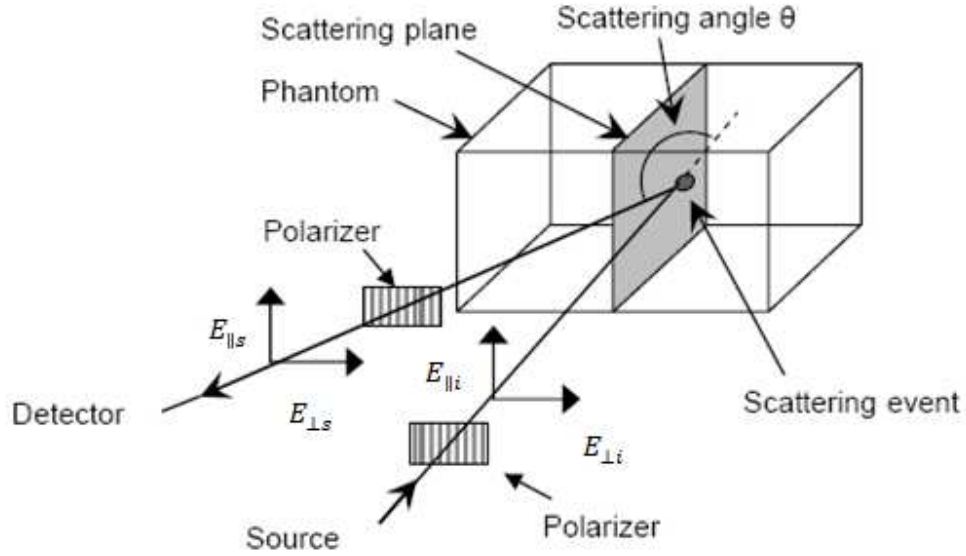


Figure 2.13. Schematic representation of single scattering event considering the incident and the scattered light component, where $E_{\parallel i}$ and $E_{\perp i}$ are the parallel and perpendicular components of incident field respectively, $E_{\parallel s}$ and $E_{\perp s}$ are the parallel and perpendicular components of scattered field respectively.

From the mathematical point of view, Mie scattering can be described briefly by considering a source, a spherical scattering particle, and an observer whose three positions

define a plane called the scattering plane. The incident and the scattered light can be reduced to their components which are parallel or perpendicular to the scattering plane, see Figure 2.13. As it is seen from the figure, the parallel and perpendicular components can be experimentally selected by a linear polarization filter oriented parallel or perpendicular to the scattering plane.

The scattering matrix describes the relationship between the incident and scattered electric field components perpendicular and parallel to the scattering plane, as the following

$$\begin{bmatrix} E_{\parallel s} \\ E_{\perp s} \end{bmatrix} = \frac{\exp(-ik(r-z))}{-ikr} \begin{bmatrix} S_2 & S_3 \\ S_4 & S_1 \end{bmatrix} \begin{bmatrix} E_{\parallel i} \\ E_{\perp i} \end{bmatrix}, \quad (2.49)$$

where $E_{\parallel i}$ and $E_{\perp i}$ are the parallel and perpendicular components of incident field respectively, $E_{\parallel s}$ and $E_{\perp s}$ are the parallel and perpendicular components of scattered field respectively, r is the distance from the scatterer.

The exponential term in Eq. (2.49), which is a transport factor, depends on the distance between the scatterer and observer. If one measures scattered light at a constant distance 'r' from the scatterer, then the transport factor becomes a constant. The total field (E_{tot}) depends on the incident field (E_i), the scattered field (E_s), and the interaction of these fields (E_{int}). In practical experiments, if one observes the scattering from a position, which avoids E_i , then both E_i and E_{int} are zero and only E_s is observed. In practical scattering measurements, Eq. (2.49) simplifies to the following,

$$\begin{bmatrix} I_{\parallel s} \\ I_{\perp s} \end{bmatrix} = const \begin{bmatrix} |S_2|^2 & 0 \\ 0 & |S_1|^2 \end{bmatrix} \begin{bmatrix} I_{\parallel i} \\ I_{\perp i} \end{bmatrix}, \quad (2.50)$$

where $I_{\parallel i}$ and $I_{\perp i}$ are the parallel and perpendicular components of incident light intensity respectively, $I_{\parallel s}$ and $I_{\perp s}$ are the parallel and perpendicular components of scattered light intensity respectively.

For unpolarized incident light, it can be shown that

$$E_s = \frac{S_{11}}{k^2 r^2} E_i, \quad (2.51)$$

where S_{11} is identical to the first element in the so-called Mueller matrix and in this case is defined as

$$S_{11} = \frac{1}{2} |S_1|^2 + \frac{1}{2} |S_2|^2. \quad (2.52)$$

2.3 Summary

Electromagnetic theory of light-tissue interaction which is taking into account individual characteristics of radiation was presented in the current chapter. There are number of computational models based on this theory. The most famous is Mie computational model for spheres which is possible to compute the scattering properties of suspension of latex spheres and tissue phantoms (Hielscher *et al.*, 1997; Kohl *et al.*, 1995). However, Mie theory has limitations that are making impossible to simulate multiple light scattering by the number of cells like in bio-tissue which is the problem of interest for us. Thus, for such purposes another theory, which is called radiative transfer theory, is usually employed and will be described in the next chapter.

Chapter 3. Radiative transfer theory

In previous chapter the individual characteristics of radiation described with the help of electromagnetic wave theory were presented. However, the way to create adequate model of light propagation within the tissues is to consider the propagation with the help of radiative transfer theory (RTT) (Case and Zweifel, 1967) because RTT is describing group and complex properties of radiation. In this chapter, the mathematical aspects of RTT in scattering media will be discussed.

The phenomena of energy transfer in different media, which is possible to absorb and scatter radiation, have been of interest for a long time (Ishimaru, 1978; Pomraning, 1973). Study of the absorption of electromagnetic radiation by the atmosphere was one of the first research in this field of interest. This problem was always essentially important for the astronomers investigating the radiation from the Sun and other stars (Howell and Siegel, 1972). The emission of gases has also been of interest for astrophysics in terms of studying the stars. Based on these investigations RTT was developed (Chandrasekhar, 1960; Sobolev, 1963; Marchuk, 1976; Thomas and Stamnes, 1999; Matzler, 2000).

RTT is considered basing on the results of Maxwell's theory of scattering on macroscopic particles (Mie scattering) (Wang and Wu, 2007). The properties of a physical medium where the process takes place are taken into account by a set of the coefficients, which are defined experimentally or by calculations (Wang and Wu, 2007).

RTT can be used for real media only with some physical assumptions (Peraiah, 2002; Wang and Wu, 2007):

- the use of the geometrical optics approximation, where the wavelength of radiation is significantly lower than the scale of variations of macro-system's parameters.
- the distance between particles essentially exceeds the radiation wavelength. The particles don't overlap each other, so there is no interference between them. The total effect

of electromagnetic field interaction with a number of particles can be represented as a sum of the interactions on each particle.

- relation of the size of particles and the wavelength is arbitrary.
- only three acts are taken into account: absorption, emission and scattering.

The fundamental difference between transfer theory and electromagnetic theory lies in the difference of description of light propagation. In classical electromagnetic theory light propagation is represented as a superposition of electromagnetic fields, however, RTT is based on the superposition of energy flux. So, in the latter case the wave properties of light (e.g. polarization, interference, and etc.) are not considered (Welch and van Gemert, 1995).

There are on the other hand also similarities between these two theories (Sudarshan, 1981; Peraiah, 2002). For example, while in electromagnetic theory the Poynting vector $\mathbf{S}$ expresses the energy transport of electromagnetic waves, in transport theory equivalent of $\mathbf{S}$ is the flux vector $\mathbf{F}$. In addition, the radiant power P transferred through a surface with the area A in electromagnetic theory,

$$P = \int_A \mathbf{S} \cdot \mathbf{n} dA, \quad (3.1)$$

can be compare with the power P ($J/s = W$) transferred through a surface with the area A within transport theory (Peraiah, 2002),

$$P = \int_A \mathbf{F} \cdot \mathbf{n} dA. \quad (3.2)$$

3.1 Transport theory

To be able to describe the propagation of light in turbid media, it is essential to explain some of the important optical parameters, which are used in modelling of light propagation in turbid media. Therefore, the propagation of photons, the radiance, fluence rate, and flux are introduced below (Welch and van Gemert, 1995; Peraiah, 2002).

3.1.1 Photon distribution function and photon power

The photon distribution function $N(r, \hat{s})$, is defined as the number of photons per unit volume moving in the direction of unit vector $\hat{s}$, in an element of solid angle containing $\hat{s}$, divided by that element, at a given point r (Welch and van Gemert, 1995). The power of the photons that propagate through the infinitesimal area dA in the infinitesimal solid angle $d\omega$ in the direction of $\hat{s}$, with velocity c_t , and energy $h\nu$ (per photon) is defined as (Welch and van Gemert, 1995)

$$dp(r, \hat{s})[Js^{-1} = W] = N(r, \hat{s})dA d\omega c_t h\nu, \quad (3.3)$$

where c_t is the velocity of light in tissue ($c_t = c_0/n$). Note that dA is perpendicular to $\hat{s}$.

3.1.2 Radiance

In framework of transport theory the propagation of light is modelled in terms of the radiance $L(r, \hat{s})$ [W/sr], Figure 3.1(a).

Radiance is the quantity used to describe the propagation of photon power with a certain direction $\hat{s}$, through a small surface dA (Welch and van Gemert, 1995),

$$L(r, \hat{s}) = \frac{dp(r, \hat{s})}{dA d\omega}, \quad (3.4)$$

Hence, it is possible to obtain the radiance by multiplying the light distribution function with the speed of light and energy of the photons in the medium as following (Welch and van Gemert, 1995),

$$L(r, \hat{s}) = N(r, \hat{s}) \frac{hc_t^2}{\lambda}, \quad (3.5)$$

where h is Planck's constant and λ the wavelength of light inside the medium.

3.1.3 Fluence rate

The fluence rate $\Phi(r)$ can be represented as the integral of the radiance over all directions, Figure 3.1(b). The advantage of usage of fluence rate in biomedical optics is the fact that it describes the propagations of photons in all direction. The fluence rate is defined as,

$$\Phi(r) = \int_{4\pi} L(r, \hat{s}) d\omega. \quad (3.6)$$

In this equation $\Phi(r)$ shows the total power incident on a small sphere at position r , divided by the crosssectional area dA of the sphere (Welch and van Gemert, 1995).

3.1.4 Net flux

The net flux is defined in terms of radiance as the sum of elemental flux according to Figure 3.1(c) (Welch and van Gemert, 1995),

$$F(r) = \int_{4\pi} L(r, \hat{s}) \hat{s} d\omega. \quad (3.7)$$

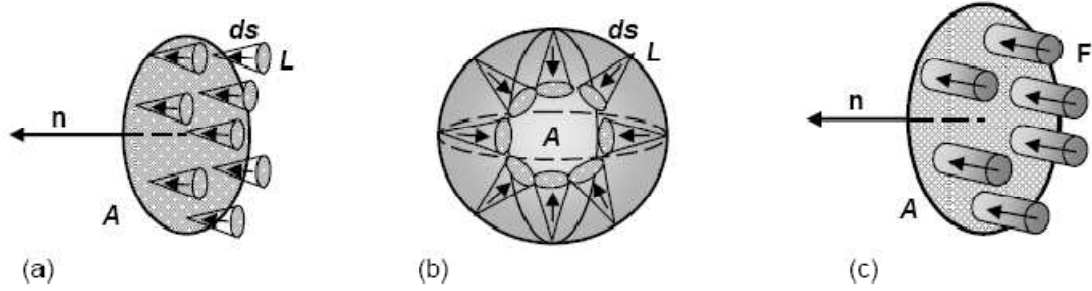


Figure 3.1. Representation of the three important optical parameter in transport theory: (a) the radiance L , (b) the fluence rate Φ , and (c) the flux F .

From Equations (3.5) and (3.7), it is possible to obtain the power incident on the right-hand side of surface A in Figure 3.1(a) (Welch and van Gemert, 1995),

$$P = \int_A \int_{2\pi} L(r, \hat{s}) (\hat{s} \cdot \hat{n}) d\omega dA. \quad (3.8)$$

Light incident on tissue is always used in different biomedical applications of lasers. So, to describe the amount of incident radiation so called irradiance E_0 [W/m^2] is usually applied. The latter value is defined as the radiant energy flux incident, on an element of the surface, divided by the area of the surface (Welch and van Gemert, 1995). There are three ways of incident light behaviour after irradiation of the tissue: one portion of radiation is reflected, and other portions are attenuated by scattering and absorption according to Beer's law:

$$\Phi(d) = E_0(1 - R)\exp(-(\mu_a + \mu_s)d), \quad (3.9)$$

where $\Phi(d)$ is the fluence rate due to the unscattered beam at position d , $\mathbf{E}_0$ is the irradiance, and R is the Fresnel surface reflection (Welch and van Gemert, 1995). The penetration depth is defined according to the total attenuation coefficient, $\mu_t = \mu_a + \mu_s$ as,

$$\delta = \frac{1}{\mu_t}. \quad (3.10)$$

3.1.5 Radiation transport equation

Let's consider photons travelling at speed c in direction $\hat{s}$ within a small volume dV . According to the law of conservation of energy the photons can only be added to or subtracted from the photon distribution function in specific interactions. Therefore, the first term of the transport equation describes the changes in the photon distribution function with time, Figure 3.2(a),

$$\int_V \frac{\partial N(r, \hat{s}, t)}{\partial t} dV, \quad (3.11)$$

where t is the time.

On the other hand, the part of the photons is lost during their penetrations through the boundaries within the tissue. The second term of the transport equation expresses such losses (Welch and van Gemert, 1995), Figure 2.2(b):

$$-\oint_S cN(r, \hat{s}, t)\hat{s}dS \text{ or } -\int_V c\hat{s}\nabla N(r, \hat{s}, t)dV. \quad (3.12)$$

The absorption of the incoming photons the direction $\hat{s}$, Figure 3.2(c), can be described as the next expression, which is the third term of the radiation transport equation:

$$- \int_V c\mu_a(r)N(r, \hat{s}, t)dV. \quad (3.13)$$

There is also scattering effect in the describing system. It is expressed with the help of the next two terms. The first one shows the photons scattering from direction $\hat{s}$ into any other direction $\hat{s}'$, Figure 3.2(d),

$$- \int_V c\mu_s(r)N(r, \hat{s}, t)dV. \quad (3.14)$$

And the second yields the photons gained through scattering from any direction $\hat{s}'$ into the direction $\hat{s}$, Figure 3.2(e)

$$\int_V c\mu_s(r) \int_{4\pi} p(\hat{s}', \hat{s})N(r, \hat{s}', t)ds'dV. \quad (3.15)$$

The last term of the radiation transport equation expresses the photons which are coming from a light source "q", Figure 3.2(f),

$$\int_V q(r, \hat{s}, t)dV. \quad (3.16)$$

Combining Equations (3.11) – (3.16) it is possible to obtain the final transport equation:

$$\int_V \frac{\partial N(r, \hat{s}, t)}{\partial t} dV = - \int_V c \hat{s} \nabla N(r, \hat{s}, t) dV - \int_V c \mu_a(r) N(r, \hat{s}, t) dV \quad (3.17)$$

$$- \int_V c \mu_s(r) N(r, \hat{s}, t) dV + \int_V c \mu_s(r) \int_{4\pi} p(\hat{s}', \hat{s}) N(r, \hat{s}', t) ds' dV + \int_V q(r, \hat{s}, t) dV.$$

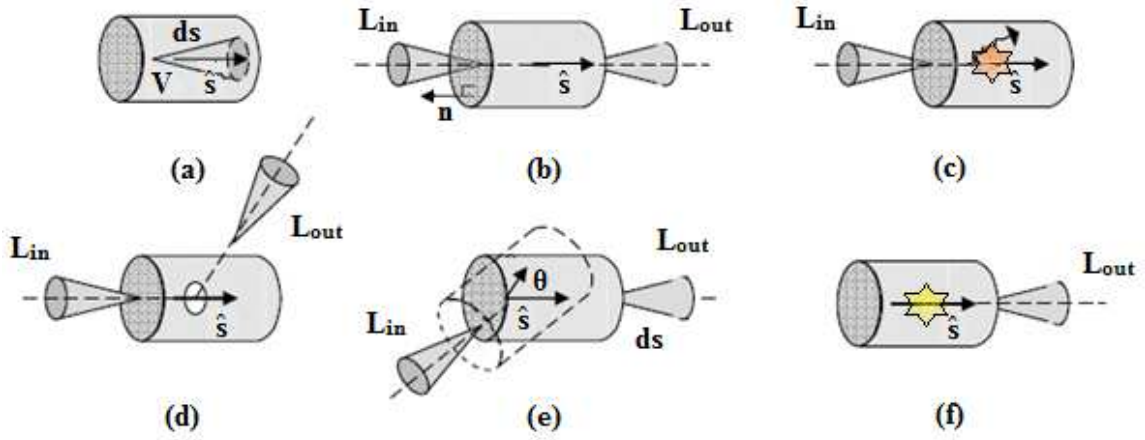


Figure 3.2. Schematic representation of the transport equation component terms. (a) The energy of the photons in volume V , (b) losses through the boundaries, (c) losses due to absorption, (d) losses due to scattering, (e) gain through scattering from any direction to direction $\hat{s}$, and (f) gain due to a light source within volume V . Here L_{in} and L_{out} are input and output radiances respectively, θ is the scattering angle.

It must be mentioned that there are few assumptions in the current analysis. First of all, the energy of the non-absorbed photons is the same during all interaction. Secondly, the scattering is elastic. And thirdly, only monochromatic radiation is taken into account here.

The time-dependent transport equation is obtained from the Eq. (3.17) by using the volume integrals:

$$\begin{aligned} \frac{1}{c} \frac{\partial L(r, \hat{s}, t)}{\partial t} + \hat{s} \nabla L(r, \hat{s}, t) + (\mu_a + \mu_s) L(r, \hat{s}, t) \\ = \mu_s \int_{4\pi} L(r, \hat{s}', t) p(\hat{s}', \hat{s}) ds' + q(r, \hat{s}, t). \end{aligned} \quad (3.18)$$

The transport equation is presented above. However, its solution should be found, because in other case it is useless. Since it is very difficult to solve the transport equation analytically, various numerical and approximation methods are employed. These methods are classified in three groups called expansion method, probabilistic method, and discretisation method.

3.2 Diffusion theory

Diffusion theory is the way of solving the transport equation from the group of expansion method. We need to find the solution of the homogeneous part of the equation and expand the general solution in terms of the obtained homogeneous solution (Case and Zweifel, 1967; Case, 1960).

In diffusion theory, the radiance (L), the phase function (p), and the source function (q) of the transport equation Eq. (3.18), are then expanded in spherical harmonics. Hence, the expansion of L can be written as (Welch and van Gemert, 1995; Peraiah, 2002),

$$L(r, \hat{s}, t) = \sqrt{\frac{1}{4\pi}} L_{00}(r, t) Y_{00}(\hat{s}) + \sqrt{\frac{3}{4\pi}} \sum_{m=-1}^1 L_{1m}(r, t) Y_{1m}(\hat{s}). \quad (3.19)$$

It is possible to find the four unknowns L_{00} and L_{1m} ($m = 0, +1, -1$) by inserting the above expression into the transport equation, Eq. (3.18), and integrating over $\hat{s}$ (Case and Zweifel, 1967).

Another approach is to observe that Y_{00} is a scalar and that Y_{1m} ($m = 0, +1, -1$) represent the three components of a vector, and thus the radiance can be expressed by them as (Case and Zweifel, 1967)

$$L(r, \hat{s}, t) = A(r, t) + \bar{B}(r, t)\hat{s}. \quad (3.20)$$

The radiance is composed of an isotropic part $A(r, t)$ and an anisotropic part $B(r, t)$ that is the linear gradient of the photon distribution function, $\hat{s}$ is the vector of gradient. The fluence rate can be obtained by inserting Eq. (3.20) into Eq. (3.6),

$$\Phi(r, t) = 4\pi A(r, t). \quad (3.21)$$

Therefore, the net flux from Eq. (3.7) can be shown as

$$\bar{F}(r, t) = \frac{4\pi}{3} \bar{B}(r, t). \quad (3.22)$$

Substituting Equations (3.21) and (3.22) in Equation (3.20), we will obtain the radiance:

$$L(r, \hat{s}, t) = \frac{1}{4\pi} (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}). \quad (3.23)$$

As it was mention above, the light source (q) is isotropic. Hence, its expansion into spherical harmonics will be

$$q(r, \hat{s}, t) = \frac{1}{4\pi} q_0(r, t). \quad (3.24)$$

Consequently, the transport equation can be written as the following (Case and Zweifel, 1967),

$$\begin{aligned} & \frac{1}{c} \frac{\partial}{\partial t} (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}) = \\ & -\hat{s} \cdot \nabla (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}) - (\mu_a + \mu_s) (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}) \\ & + \mu_s \int_{4\pi} p(\hat{s}', \hat{s}) (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}) ds' + q_0(r, t). \end{aligned} \quad (3.25)$$

Considering the scattering phase function Eq. (3.25) is reduced to

$$\begin{aligned} & \frac{1}{c} \frac{\partial}{\partial t} (\Phi(r, t) + 3\bar{F}(r, t) \cdot \hat{s}) = -(\hat{s} \cdot \nabla + \mu_a) \Phi(r, t) \\ & -3(\hat{s} \cdot \nabla + \mu_a + \mu_s') \bar{F}(r, t) \cdot \hat{s} + q_0(r, t). \end{aligned} \quad (3.26)$$

If we integrate this expression we will obtain:

$$\frac{1}{c} \frac{\partial \Phi(r, t)}{\partial t} = -\mu_a \Phi(r, t) - \nabla \cdot \bar{F}(r, t) + q_0(r, t). \quad (3.27)$$

On the other hand, if Eq. (3.26) is multiplied by $\hat{s}$ and then the integration is carried out, it results in (Case and Zweifel, 1967)

$$\frac{1}{c} \frac{\partial \bar{F}(r, t)}{\partial t} = \frac{1}{3} \nabla \Phi(r, t) - (\mu_a + \mu_s') \bar{F}(r, t). \quad (3.28)$$

The Equations (3.27) and (3.28) are the basic equations of diffusion theory.

By eliminating the dependent variable F , these two equations can be reduced to one equation. The steady-state solution of Eq. (3.28) presents Fick's law (Case and Zweifel, 1967),

$$\bar{F}(r, t) = -D(r) \nabla \Phi(r, t), \quad (3.29)$$

where the diffusion coefficient D is expressed as,

$$D(r) = \frac{1}{3(\mu_a + \mu_s')}. \quad (3.30)$$

Hence, the time-dependent diffusion equation is obtained as:

$$\frac{1}{c} \frac{\partial \Phi(r, t)}{\partial t} - D(r) \nabla^2 \Phi(r, t) + \mu_a \Phi(r, t) = q_0(r, t). \quad (3.31)$$

It should be mentioned that the diffusion equation is valid only if the propagating light is diffused. Thus, the reduced scattering coefficient is much larger than the absorption, i.e.

$$\mu_s' \gg \mu_a. \quad (3.32)$$

Another assumption is that the light must be diffused when it reaches the detector. Consequently, the source and the detector need to be separated in space and time. It has been shown that diffusion theory is inaccurate in applications that involve structures near

the surface of the medium, small source-detector distances, and geometrical configurations with sharp discontinuities of the scattering properties (Wang and Jacques, 2000).

3.2.1 Time-domain and point source solutions

Let's consider an infinite homogeneous medium or a medium with simple boundary conditions. And let the source in that media will be a short pulse isotropic point source, i.e.

$$q_0(r, t) = \delta(0,0), \quad (3.33)$$

where δ is Dirac's delta function.

For that case the solution of the diffusion equation is a Green's function expressed as (Case and Zweifel, 1967; Peraiah, 2002),

$$\Phi(r, t) = Q_s c (4\pi Dct)^{-3/2} \exp\left(-\frac{r^2}{4Dct} - \mu_a ct\right), \quad (3.34)$$

where Q_s is the energy of the short pulse from the point source at ($t = 0$) and r is the radial distance from the source.

However, the source function can be described by an isotropic point source only in case of illuminating of a point on the tissue surface. In the case of illuminating of the small spot it is possible to use the set of isotropic point sources with an exponentially decay strength, proportional to $\exp[-(\mu_a + \mu_s')z]$, where z shows the distance from the boundary. Thus, the fluence rate can be calculated from an integral of Green's function for sources at all depths (Case and Zweifel, 1967).

The representation of the case of isotropic scattering can be simplified by assuming that all the incident photons are initially scattered at a depth equal to the mean free path ($z = 1/$

μ_s'), Figure 3.3. Here, source can be approximated with the simple delta-function, as the following (Patterson *et al.*, 1989),

$$q_0(r, t) = \delta(r - r_0)\delta(t). \quad (3.35)$$

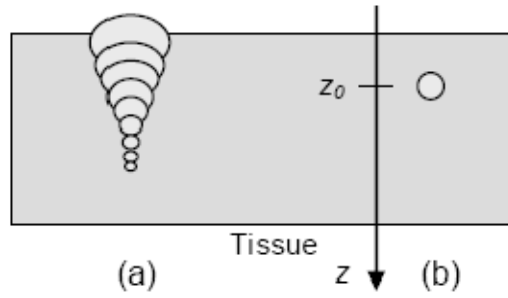


Figure 3.3. Schematic representation of the two possible configurations of the point source. (a) A continuous line of sources with an exponential decay, and (b) the source at a depth of $z = 1/\mu_s'$, represented by delta function, z_0 is the initial position of the point source.

The boundary conditions must be considered while solving diffusion equation. There should be no photon flux back into the turbid medium at the surface when the refractive indices are matched. A good approximation is to introduce a virtual, or extrapolated boundary ($z = z_e$), and there apply $\Phi = 0$ (Farrel *et al.*, 1992). For index matching, one can derive the value $z_e \approx 2D$ (Ishimaru, 1978). In case of refractive index mismatching there will be an internal reflection at the boundary and the latter distance will change (Farrel *et al.*, 1992; Haskell *et al.*, 1994). For $n = 1.4$, an extrapolated boundary at $z_e \approx 5.5D$ is appropriate. The method of images or negative mirror sources, is introduced to set the fluence to zero at the extrapolated boundary (Haskell *et al.*, 1994). As it is shown in Figure 3.4, the point source $z = z_0$, is mirrored in $z = -z_e$, resulting in a negative source at $z = -(2z_e + z_0)$.

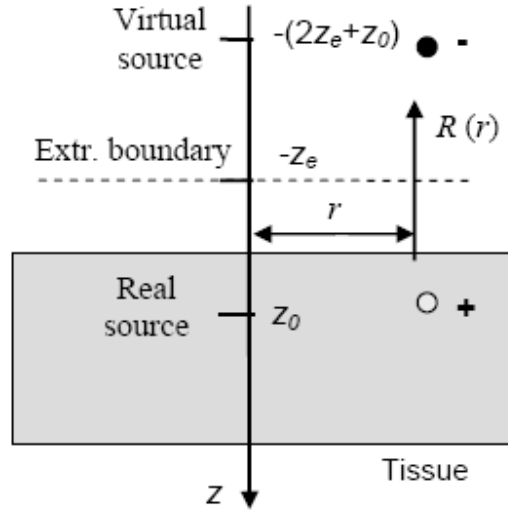


Figure 3.4. Schematic picture showing the principle of an extrapolated boundary at $z = -z_e$, with $\Phi = 0$. A dipole source is placed symmetrically around the extrapolated boundary, with a positive part at $z = z_0$, inside the medium, and a negative part at $z = -(2z_e + z_0)$.

If the observation of the radiance is made at a distance from the source that is considerably larger than the extrapolated boundary length, the zero radiance at the physical boundary is a valid approximation; but at too short distances, the diffusion approximation is not valid.

For a semi-infinite homogeneous media it is possible to obtain simple solutions. The time resolved diffuse reflectance is expressed as the following (Patterson *et al.*, 1989)

$$R(r, t) = \frac{1}{2} (4\pi Dc)^{-3/2} t^{-5/2} \exp(-\mu_a ct) \left[z_0 \exp\left(-\frac{r_1^2}{4Dct}\right) + (z_0 + 2z_e) \exp\left(-\frac{r_2^2}{4Dct}\right) \right] \quad (3.36)$$

The corresponding steady-state diffuse reflectance is expressed as

$$R(r) = \left[\frac{1}{4\pi} z_0 \left(\mu_{eff} + \frac{1}{r_1} \right) \frac{\exp(-\mu_{eff} r_1)}{r_1^2} + (z_0 + 2z_e) \left(\mu_{eff} + \frac{1}{r_2} \right) \frac{\exp(-\mu_{eff} r_2)}{r_2^2} \right], \quad (3.37)$$

where

$$\mu_{eff} = \sqrt{\frac{\mu_a}{D}}, r_1 = \sqrt{z_0^2 + r^2}, r_2 = \sqrt{(z_0 + 2z_e)^2 + r^2}, z_0 = \frac{1}{\mu_s}, z_e \approx bz_0. \quad (3.38)$$

Here, " b " is dependent on the actual Fresnel reflection coefficient, for example, $b = 2$ for a typical tissue-air boundary (Peraiah, 2002).

The total diffuse light which is transmitted through a homogeneous sample is detected on the opposite side to the source and can be represented as

$$T(d, t) = (4\pi Dc)^{-1/2} t^{-3/2} \exp(-\mu_a ct) \left[(d - z_0) \exp\left[-\frac{(d - z_0)^2}{4Dct}\right] + (d + z_0) \exp\left[-\frac{(d + z_0)^2}{4Dct}\right] + (3d - z_0) \exp\left[-\frac{(3d - z_0)^2}{4Dct}\right] - (3d + z_0) \exp\left[-\frac{(3d + z_0)^2}{4Dct}\right] \right] \quad (3.39)$$

where " d " is the thickness of the slab.

Fitting the analytical solutions of Eqs. (3.36) and (3.39) to a measured reflectance or transmittance curve, will provide an estimate of optical properties μ_a and μ_s' . The g -value can never be evaluated with diffusion theory.

3.2.2 Frequency domain solutions

Sinusoidal modulated light source with frequency f is used to launch photons into the tissue in case of frequency domain (Welch and van Gemert, 1995). There are two components of source fluence rate: $AC(\Phi_0)$ and $DC(\Phi_1)$. Thus, the photon density waves are generated, which are propagating within the considering medium with rapidly decaying DC and AC amplitudes. These photon density waves are describing with the net fluence rate which can be represented with the following expression (Haskell *et al.*, 1994, Tromberg *et al.*, 1993):

$$\begin{aligned}\Phi(r, t) &= DC(r) + AC(r)\exp[i(k_i - \omega t)] \\ &= \Phi_0 \frac{\exp(-\mu_{eff}r)}{r} + \frac{\exp(-k_r r)}{r} \exp[i(k_i r - \omega t)],\end{aligned}\quad (3.40)$$

where

$$\mu_{eff} = \left(\frac{\mu_a}{D}\right)^{1/2} = (3\mu_a(\mu_a + \mu_s'))^{1/2}. \quad (3.41)$$

In comparison between light propagation in time domain and in frequency domain it can be said that in applying short pulse to a scattering medium, this pulse is broadened in time due to propagating to many paths between source and detector. Then, the observed quantity is the number of photons reaching the detector per unit time at a given time, $h(t)$. However, in frequency domain, applying the sinusoidally modulated light source, the observed quantity at the detector, are the phase angle between the detector and the source, and the amplitude of the oscillation relative to the DC level. In this case, the photon flux at the detector will be sinusoidal in time but the oscillation will be delayed in time relative to the source and reduced in amplitude relative to the average flux. The time domain signal $h(t)$ is linked to the modulation and phase by Fourier transformation so that any information

obtained in the time domain can be obtained in the frequency domain, as well (Welch and van Gemert, 1995). However, there are a few advantages of using frequency domain rather than time domain sources such as narrow band and noise resistant measurements in frequency domain measurements.

3.3 Summary

Radiative transport theory as a method of description of group complex characteristics of radiation was presented in the current chapter. The equation of transport is equivalent to the equation of Boltzmann's kinetic theory of gases and to the equation of neutron transport theory (Case and Zweifel, 1967). RTT is so called deterministic model and is possible successfully describe the transport of photons within the medium. However, there are some difficulties in application of this model. Although RTT is computationally fast there can be losses in accuracy especially in cases of inhomogeneities in biotissues or structures with complex geometry. Additionally, coherence properties cannot be taken into account with RTT. Therefore, it is essentially important for our purposes to employ Monte Carlo method which represents stochastic models and is possible to avoid difficulties listed above.

Chapter 4. Monte Carlo simulation as a computer method of Radiative Transfer Equation solution

4.1 Principles of Monte Carlo method

The Monte Carlo (MC) method is rapidly becoming the model of choice for simulating light transport in tissue. Principles of the simulation of optical/laser radiation propagation in biological media by MC technique are described widely (Bonner *et al*, 1987; Keijzer *et al*, 1989; Prahl *et al*, 1989; Meglinski and Matcher, 2001; Churmakov, 2005). Thus, the authors' aim was not to develop MC model again but basing on the existing computational model, which was developed in Cranfield University (Churmakov, 2005), improve it with including of the coherence properties of light and polarization in the MC code.

In MC technique, every simulation is based upon events that happen randomly, and so the outcome of a calculation is not always absolutely predictable. This element of chance reminds one of gambling, so the originators of the Monte Carlo technique, Ulam and von Neumann, called the technique Monte Carlo to emphasize its gaming aspect. MC simulations can be used for simulating all kinds of particle transport. Mainly it has been developed for neutron transport in combination with nuclear reactions.

In tissue optics, MC modelling is used as a technique for describing light transport in tissue, based on the transport equation and the random walk of photons in absorbing and scattering medium. In this case, the photons are considered as neutral particles and all wave phenomena are neglected.

In MC simulation, a photon package is traced through the tissue until it exits or is terminated due to absorption. Many physical parameters of the photon package such as the distribution of absorption, exiting position, time-of-flight, etc can be logged. By repeating the tracking process for a large number of photon packages, it is possible to obtain statistics for these physical quantities. In MC simulations, the key decisions are the mean free path for scattering or absorption events, and the scattering angle. The number of photons needed

depends on the problem and the wanted accuracy. Another aspect of the MC simulations is that they are based on macroscopic optical properties (μ_a , μ_s , and g) that are assumed to extend uniformly over small units of tissue.

The main advantage of MC method is that there is no limitation concerning boundary conditions or spatial localization of inhomogeneties in the tissue. On the other hand, the main drawback of MC is the problem of getting good statistics, particularly if the point of interest is located far away from the point of entry of the light and the scattering and absorption coefficients are high. For sampling the random variable x in MC simulation, a probability density $p(x)$ is considered, which defines x over the interval $a \leq x \leq b$ such that:

$$\int_a^b p(x)dx = 1. \quad (4.1)$$

The probability that x will fall in the interval $[a, x]$ is given by the distribution function $F_x(x_1)$ as the following,

$$F_x(x_1) = \int_a^{x_1} p(x)dx. \quad (4.2)$$

By using a random number generator, one can obtain a random number ζ in the range of $[0,1]$. The probability density function for this random number is 1 in the range $[0,1]$, and the corresponding probability distribution is as the following,

$$F_\zeta(\zeta_1) = \int_a^{\zeta_1} p(\zeta)d\zeta = \zeta_1, \quad 0 \leq \zeta_1 \leq 1. \quad (4.3)$$

This means that the picked random number gives the integrated value of $p(x)$, which is the basic equation for sampling random variables from non-uniform distribution using uniformly distributed random number. This equation is defined as

$$\zeta = F_x(x) = \int_a^{x_1} p(x)dx. \quad (4.4)$$

The simulation is based on the modelling of large number of possible trajectories of the photon packets from the source to the detector (Figure 3.1).

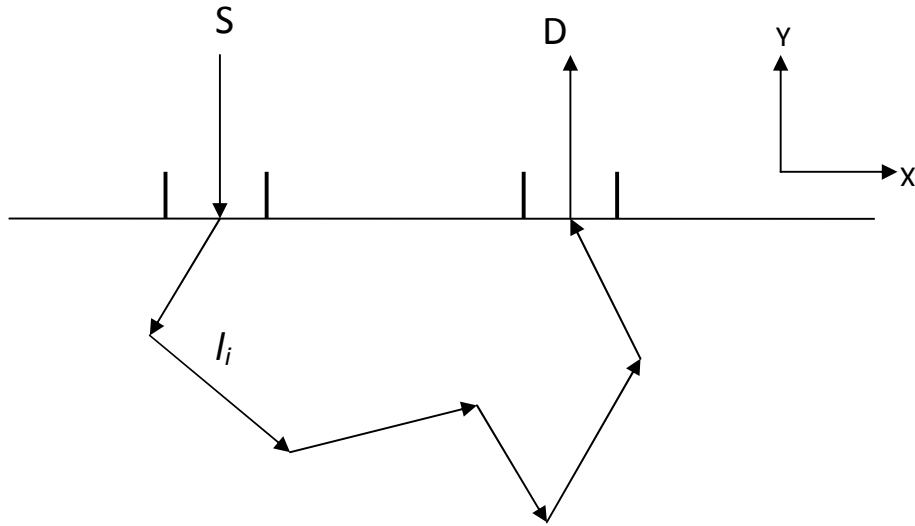


Figure 4.1. Schematic presentation of Monte Carlo method. S and D are the source and detector respectively. l_i is the photon packet pathlength between i -th and $i+1$ -th scattering events.

Modelling of an individual photon packets trajectory consists with a sequence of the following simulations: photon pathlength generation, scattering events and reflections/refractions on the medium boundary. The initial and final states of the photons are determined by the source and the detector geometries and numerical apertures.

Photon pathlength l can take any positive values with the probability density function:

$$p(l) = \mu_t \exp(-\mu_t l), \quad (4.5)$$

where $\mu_t = \mu_s + \mu_a$ is the extinction coefficient, μ_s and μ_a are the scattering and absorption coefficients respectively. Independence of the absorption and scattering in the medium allows simulating them separately. So, the pathlength of a photon is modelled as:

$l_i = -\frac{\ln(\xi)}{\mu_s}$, where ξ is a uniformly distributed random number between 0 and 1.

Following earlier works (Flock *et al*, 1989; Keijzer *et al*, 1989) we use homogeneous phase function, relative to the mutual orientation of vectors $\mathbf{u}$ and $\mathbf{v}$, which are the photon packet directions before and after the scattering respectively. This function is known as a Henyey-Greenstein scattering phase function:

$$p(\mathbf{u}, \mathbf{v}) = p(\cos \theta) = \frac{1}{4\pi} \frac{(1 - g^2)}{(1 + g^2 - 2g \cos \theta)^{3/2}}, \quad (4.6)$$

where g is the anisotropy factor. To make the progress of coordinates smoother we convert angles to their direction cosines. Initially photon packets are emitted perpendicular to the medium surface, so the initial direction cosines represent as $u_1 = 0$, $u_2 = 0$ and $u_3 = 1$. New values for the directional cosines, v_1, v_2, v_3 are calculated as:

$$\begin{pmatrix} v_1 \\ v_2 \\ v_3 \end{pmatrix} = \begin{bmatrix} \frac{1}{\sqrt{1-u_3^2}} u_1 u_3 & -\frac{1}{\sqrt{1-u_3^2}} u_2 & u_1 \\ \frac{1}{\sqrt{1-u_3^2}} u_2 u_3 & \frac{1}{\sqrt{1-u_3^2}} u_1 & u_2 \\ -\sqrt{1-u_3^2} & 0 & u_3 \end{bmatrix} \begin{pmatrix} \sin \theta \cos \varphi \\ \sin \theta \sin \varphi \\ \cos \theta \end{pmatrix}. \quad (4.7)$$

The specular and internal reflection on the medium boundary is taken into account by splitting the photon packet into the reflected and the transmitted parts, the weights of which are attenuated as:

$$W = (1 - R(\theta_i))(1 - R_{in})W_0 \left[\prod_{p=1}^M R(\theta_i) \right], \quad (4.8)$$

where W_0 is the initial weight of the photon packet, M is the number of photon packet reflections/refractions on the medium boundary, R_{in} is the Fresnel coefficient relating to the initial reflection on the medium boundary surface, when photon packet enters the medium, $R(\theta_i)$ is the Fresnel reflection coefficient.

The photon packet tracing is generated until its escaping at the detector or leaving the medium. The simulation of a photon packet tracing is stopped when its statistical weight is less than 10^{-3} , or a photon packet has been scattered more than 10^4 times.

4.2 Assumptions used in the modelling

Let's represent the field of incident low-coherent optical radiation in the form of the plane-wave spectral decomposition

$$E_0(\mathbf{r}, t) = \int_{-\infty}^{\infty} d\omega f(\omega - \omega_0) \exp(-i\omega t + i\mathbf{k} \cdot \mathbf{r}). \quad (4.9)$$

Here, ω is the frequency and $\mathbf{k}$ is the wave vector satisfying the dispersion relation

$$k^2 c_0^2 - n(\omega) \omega^2 = 0, \quad (4.10)$$

where $n(\omega)$ is the refractive index of the medium disregarding random inhomogeneities or scatterer particles and c_0 is the speed of light in vacuum. We assume that the spectral distribution $f(\omega - \omega_0)$ is Gaussian with the half-width Ω and centre at the frequency ω_0 :

$$f(\omega - \omega_0) = \frac{1}{\Omega} \exp \left[-\frac{(\omega - \omega_0)^2}{2\Omega^2} \right]. \quad (4.11)$$

Disregarding the dispersion of the refractive index in the frequency band $\omega_0 \pm \Omega$, the wavenumber can be represented as

$$k \approx \frac{n(\omega_0)\omega}{c_0}, \quad (4.12)$$

where $n(\omega_0)$ is the refractive index at the frequency ω_0 .

When describing laser radiation pulses in the frequency space, the FWHM $\Delta\nu$ of the frequency spectrum of pulse intensity is used instead of the half-width of the Gaussian in units of cyclic frequency Ω :

$$\Omega = (\ln 2)^{-1/2} \pi \Delta\nu. \quad (4.13)$$

The coherence length l_c of incident radiation is defined as the total length of the pulse in which it is halved:

$$l_c = \frac{2 \ln 2}{\pi} \frac{c}{\Delta\nu}, \quad (4.14)$$

or

$$l_c = \frac{2 \ln 2}{\pi} \frac{\lambda^2}{\Delta \lambda}. \quad (4.15)$$

Here, $\Delta \lambda$ is the FWHM of the wavelength spectrum.

When investigating the multiple scattering effects, only a scalar field is usually considered with the change of the Maxwell wave equation to the Helmholtz equation. The scalar field $E(\mathbf{r}, t)$ scattered by dielectric-constant inhomogeneities $\Delta \varepsilon(\mathbf{r})$ in the random medium is described by the wave equation

$$E(\mathbf{r}, t) = E_0(\mathbf{r}, t) + \int d\mathbf{r}' dt' T(\mathbf{r} - \mathbf{r}', t - t') \Delta \varepsilon(\mathbf{r}') E(\mathbf{r}', t'), \quad (4.16)$$

where

$$T(\mathbf{r}, t) = \frac{k^2}{4\pi r} \exp\left(-\frac{r}{2l}\right) \delta\left(t - \frac{r}{c}\right), \quad (4.17)$$

is the space-time Green's function in the homogeneous medium with the complex refractive index $n(\omega_0)$, $\Delta \varepsilon(r)$ is the dielectric-constant fluctuations, c is the speed of light in the medium,

$$l = \left(2 \operatorname{Im} \left(\frac{n(\omega_0) \omega_0}{c_0} \right) \right)^{-1}, \quad (4.18)$$

is the photon mean free path. Integrating the wave equation, the random field can be represented in the form of the series

$$E(\mathbf{r}, t) = E_0(\mathbf{r}, t) + \sum_{n=1}^{\infty} \int \prod_{i=1}^n \Delta \varepsilon(\mathbf{r}_i) d\mathbf{r}_i T_s(\mathbf{r} - \mathbf{r}_n) \dots T_s(\mathbf{r}_2 - \mathbf{r}_1) \times E_0\left(\mathbf{r}_1, t - \frac{|\mathbf{r} - \mathbf{r}_n| + R_n}{c}\right), \quad (4.19)$$

where

$$T_s(\mathbf{r}) = \frac{k^2 \exp(ikr)}{r}, \quad (4.20)$$

is the static field Green's function and

$$R_n = \sum_{1 \leq j \leq n} |\mathbf{r}_j - \mathbf{r}_{j+1}|, \quad (4.21)$$

is the optical path length between the points $\mathbf{r}_1$ and $\mathbf{r}_n$ including n scattering events.

The averaging of the product of the field by the complex conjugate field over the scatterer configurations yields the mean intensity

$$I(\mathbf{r}, t) = I_0(\mathbf{r}, t) + I_{sc}(\mathbf{r}, t). \quad (4.22)$$

Here,

$$I_0(\mathbf{r}, t) = |\langle E(\mathbf{r}, t) \rangle|^2, \quad (4.23)$$

is the non-fluctuating intensity component and

$$I_{sc}(\mathbf{r}, t) = \langle \delta E^*(\mathbf{r}, t) \delta E(\mathbf{r}, t) \rangle, \quad (4.24)$$

is the scattered radiation intensity, where

$$\delta E(\mathbf{r}, t) = E(\mathbf{r}, t) - \langle E(\mathbf{r}, t) \rangle, \quad (4.25)$$

is the field fluctuations.

When averaging over the random inhomogeneities, the terms of products of series contain multiparticle average fluctuations of the permittivity. Owing to randomization in the phase difference appearing in multiple scattering, only the incoherent component (ladder in diagram terms), which describes the sequences of scattering events for fields δE and δE^* for the same sequence of dielectric-constant fluctuations, remains. As a result, the scattered radiation intensity at the observation point $\mathbf{r}_d$ at instant t_d can be represented as the multiple scattering series

$$I_{sc}(\mathbf{r}_d, t_d) = \sum_{n=1}^{\infty} I_n(\mathbf{r}_d, t_d). \quad (4.26)$$

The n th-order scattering intensity contribution has the form

$$I_n(\mathbf{r}_d, t_d) = \left(\frac{\omega_0}{c_0} \right)^{4n} \times \prod_{j=1}^n \int d\mathbf{r}_j G(\mathbf{q}_j) \Lambda(\mathbf{r}_{j+1} - \mathbf{r}_j) I_0(\mathbf{r}_1, t_1), \quad (4.27)$$

where the Fourier transform of the correlation function of dielectric-constant fluctuations,

$$\tilde{G}(\mathbf{q}) = \int \langle \Delta \varepsilon(\mathbf{r}_1) \Delta \varepsilon^*(\mathbf{r}_2) \rangle \exp[-i\mathbf{q}(\mathbf{r}_1 - \mathbf{r}_2)] d\mathbf{r}_2, \quad (4.28)$$

is the differential cross section for single scattering up to the normalization factor. The vector $\mathbf{q}_j$ describes change in the wave vector at the point $\mathbf{r}_j$ of the j -th scattering event; $\mathbf{r}_{n+1}$ for n scattering events coincides with the observation point, $\mathbf{r}_{n+1} = \mathbf{r}_d$; and

$$I_0(\mathbf{r}_1, t_1) \propto \exp\left(-\Omega^2 \left(t - \frac{r}{c}\right)^2\right) = \exp(-4\ln 2 (ct - r)^2 l_c^{-2}), \quad (4.29)$$

is the incident radiation intensity. The function

$$\Lambda(\mathbf{r}_1 - \mathbf{r}_2) = \frac{\exp[-|\mathbf{r}_1 - \mathbf{r}_2|/l]}{|\mathbf{r}_1 - \mathbf{r}_2|}, \quad (4.30)$$

describes the propagation of intensity between two sequential scattering events. The instant t_1 determines the time necessary for a ray undergoing n scattering events to arrive at the observation point at instant $\mathbf{r}_d$:

$$t_1 = t_d - \left(|\mathbf{r}_d - \mathbf{r}| + |\mathbf{r}_1| + \frac{R_n}{c}\right). \quad (4.31)$$

In multiple scattering problems, of key importance is the optical theorem

$$\left(\frac{\omega_0}{c_0}\right)^4 \int d\sigma^2 \tilde{G}(\mathbf{k}_2 - \mathbf{k}_1) = l_s^{-1}, \quad (4.32)$$

which relates the scattering length l_s and cross section for single scattering. The photon mean free path l and scattering length l_s are related as

$$\frac{1}{l} = \frac{1}{l_s} + \frac{1}{l_a}, \quad (4.33)$$

where l_a is the absorption length due to inelastic scattering.

4.3 Summary

MC method of simulation of light propagation within the complex media was presented above. As the basis was taken existing computational model, which has been developed in Cranfield University (Churmakov, 2005). It was improved with including of the coherence properties of light and polarization in the MC code. These reflected on the accuracy of modelling and increased value of the code for the optimization of the experimental OCT setups. The results of MC modelling are presented in the sixth chapter.

Chapter 5. Low-coherence optical techniques and their application

5.1 Optical Coherence Tomography (OCT)

Optical Coherence Tomography (OCT) is hot topic of research for a few decades (Huang *et al.*, 1991). Low coherence interferometry (LCI) lies in the basis of OCT. Principles of LCI were firstly introduced by Sir Isaac Newton and later again by Fercher *et al.*, Danielson *et al.*, Youngwist *et al.*, and Takada *et al.*, among others (Danielson *et al.*, 1987; Fercher, 1986; Fujimoto, 2002; Fujimoto, 2003; Masters, 1999; Takada *et al.*, 1987; Younguist *et al.*, 1987; Kubota *et al.*, 1987).

OCT is an imaging technique analogous to ultrasound. The difference is in the employed sources of signal. In ultrasound techniques the difference in the flight times of acoustic waves is used to obtain the structural images of the tissue. At the same time, OCT employs low time-coherence infrared light and are working on the basis of Michelson's interferometer to get relatively high resolution image. Figure 5.1 schematically represents typical structure of interferometer which is used in OCT.

The description of the OCT principles is possible with the help of the theory of two-beam interference for partially coherent light. The light is generated with a light source and subsequently splitted with a beam splitter (BS) 50/50 to the reference (RA) and sample (SA) arms respectively. Assumption of the sample (Fig. 5.1) as a perfectly reflecting mirror and ignoring of the polarization effects allows us to consider the scalar complex functions $E_s(t - L_s/c)$ and $E_r(t - L_r/c)$, where L_r and L_s – optical path lengths in the reference and sample arms respectively, and c is the speed of light, which are describing the sample beam and the reference beam respectively.

The resultant intensity I can be presented as (Fercher *et al.*, 2003)

$$I(\tau) = \langle [E_s(t) + E_r(t + \tau)][E_s(t) + E_r(t + \tau)]^* \rangle, \quad (5.1)$$

where $\tau = \Delta L/c$ is the time delay.

The time delay appears according to the round-trip optical path length difference between the two arms.

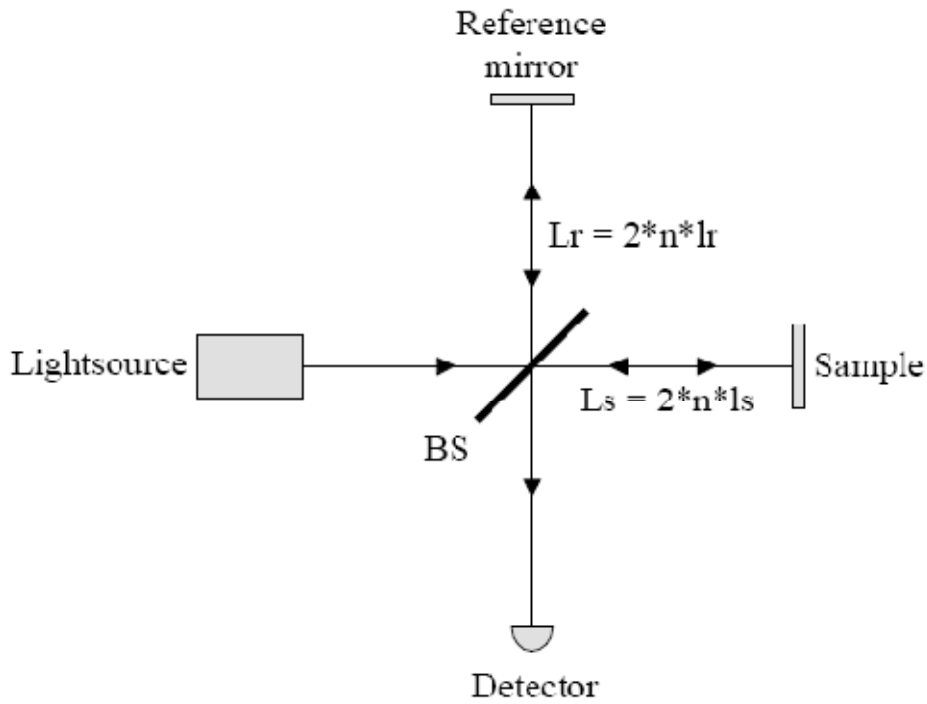


Figure 5.1. Schematic representation of a Michelson interferometer used in OCT. BS – beam splitter, L_r and L_s – optical path lengths in the reference and sample arms respectively; l_s and l_r – the geometric lengths of the arms; n – the refractive index. Figure was adopted from Fercher et al., 2003.

Let's describe intensities I_s and I_r for both sample and reference components:

$$I_s = \langle E_s(t) E_s^*(t) \rangle, \quad (5.2)$$

$$I_r = \langle E_r(t + \tau)E_r^*(t + \tau) \rangle. \quad (5.3)$$

Hence, the resultant intensity can be shown as

$$I(\tau) = I_s + I_r + 2\sqrt{I_s I_r} \text{Re}(V(\tau)). \quad (5.4)$$

The last term of the Eq. (5.4) represents the amplitude of the interference fringes, which depends on the optical path delay set by the position of the reference mirror and which allows to obtain the information about the internal structure of the observed specimen. $V(\tau)$ is the normalized coherence function and it can be written as (Fercher *et al.*, 2003)

$$V(\tau) = \frac{\langle E_s(t)E_r^*(t + \tau) \rangle}{\sqrt{I_s I_r}}. \quad (5.5)$$

The optical path delay creates phase difference which is represented as

$$\varphi = 2\pi f_0 \tau, \quad (5.6)$$

where f_0 - the central frequency of the light source. Assuming the fact that E_s and E_r originate primarily from a single wave front resulting intensity can be rewritten into the form (Pan *et al.*, 1995; Schmitt, 1999; Wang *et al.*, 2004):

$$I(\tau) = I_s + I_r + 2\sqrt{I_s I_r} |V_t(\tau)| \cos(2\pi f_0 \tau). \quad (5.7)$$

If the process of radiation of light is stationary and ergodic the temporal coherence function $V_t(\tau)$ is the Fourier transform of the power spectral density $S(f)$ of the light source

according to the Wiener-Khintchine theorem (Akçay *et al.*, 2002; Gilgen *et al.*, 1989; Schmitt, 1999; Wang, 1999):

$$V_t(\tau) = \int_0^{\infty} S(f) \exp(-i2\pi\tau) df. \quad (5.8)$$

Eq. (5.8) shows us that sensitivity of the LCI to the optical path difference of the sample and reference arm depends on the characteristics of the emission spectrum of the light source. Thus, it is possible to write the next equation:

$$I(\Delta L) = I_s + I_r + 2\sqrt{I_s I_r} |V_t(\Delta L)| \cos(k_0 \Delta L), \quad (5.9)$$

where $k_0 = 2\pi/\lambda_0$ is the average wave number; $\lambda_0 = c/f_0$.

Presented above equation is correct only in case of the perfectly reflecting mirror as a sample. However, the real conditions suppose that reflected from the specimen light can be divided on the case of the backscattered light, that undergoes single or just few scattering events, and on the case of multiple scattering.

Taking into account the scattering path inside the media we will obtain (Wang and Wu, 2007)

$$L_s = L_{s0} + \sum n_s l_i, \quad (5.10)$$

where L_{s0} is the round-trip path length to the sample surface; n_s - the refractive index of the medium; l_i - the scattering path of light inside the medium. Then the intensity given in Eq. (5.7) can be written in the form (Wang and Wu, 2007)

$$I(\tau) = \left\langle \left[\int_{L_{s0}}^{\infty} E'_s(t, L_s) dL_s + E_r(t + \tau) \right] \times \left[\int_{L_{s0}}^{\infty} E'_s(t, L_s) dL_s + E_r(t + \tau) \right]^* \right\rangle, \quad (5.11)$$

where $E'_s(t, L_s)$ - the path length-resolved field density.

$$I(L_s, L_r) = I_s + I_r + 2\sqrt{I_s I_r} \int_{-\infty}^{\infty} \sqrt{R(L_s)} |V_t(\Delta L)| \cos(k_0 \Delta L dL), \quad (5.12)$$

where $R(L_s)$ – the normalized path length-resolved diffuse reflectance.

The signal-carrying interference term can be expressed as a convolution (Fercher *et al.*, 2003),

$$I_{sign}(L_s, L_r) = 2\sqrt{I_s I_r} [\sqrt{R(L_s)} \otimes C(L_s - L_r)], \quad (5.13)$$

where $C(L_s - L_r)$ - the coherence function. It is also called a point spread function PSF of the light source. $\otimes$ denotes the convolution operation. As a conclusion, low coherence interferometry traces out variation in the path length-resolved reflectances defined by $R(L_s)$ (Kulkarni *et al.*, 1997; Pan *et al.*, 1995; Pan *et al.*, 1996; Schmitt, 1999; Yung *et al.*, 1999).

5.1.1. Main characteristics of OCT

Among the main physical properties of OCT it is possible to determine the axial resolution and the transverse resolution. The first one depends on the properties of the light source and the second one on the optics of the imaging device.

The axial resolution is characterized with the coherence length L_c . If autocorrelation function has Gaussian shape, it is possible to present coherence length as:

$$L_c = \frac{2 \ln 2}{\pi} \frac{\lambda_0^2}{\Delta \lambda}, \quad (5.14)$$

where $\Delta \lambda$ is the FWHM (full width at half maximum) wavelength range of the light source (Brezinski, 1999). Eq. (5.14) is limited with the assumption that concerned medium is vacuum. However, the real refractive index of the sample depends on the wavelength of light within medium. The impact of that is increasing especially when broadband sources are used (Sampson, 2004). The group refractive index n_g depends on the wavelength. As a result the coherence length is increasing which leads to the reduction of the resolution consequently. Thus, in a dispersive medium the coherence length L_c can be presented as:

$$L_c = \sqrt{L_c^2 + \left(\frac{dn_g}{d\lambda} d_g \Delta \lambda \right)^2}, \quad (5.15)$$

where d_g is the geometric depth in the medium (Drexler *et al.*, 1998; Hitzenberger *et al.*, 1998; Hitzenberger *et al.*, 1998; Hitzenberger *et al.*, 1999).

The transversal resolution is determined only by the optics of the imaging device and, hence, doesn't correlate with the axial resolution. The transversal resolution Δx can be written as

$$\Delta x = \frac{4\lambda}{\pi} \left(\frac{f_1}{d} \right), \quad (5.16)$$

where f_1 is the focal length of the focusing lens and d is the light beam diameter on the lens aperture.

5.1.2. Applications of OCT in biomedicine

The OCT is very promising diagnostic technique due to its non-invasiveness in the measurement of bio-tissues morphology and properties because of the usage of the electromagnetic radiation as a probing agent. The understanding of how to obtain the required information from the sample and how to improve and develop modalities of OCT became possible because of the understanding how the radiation interact with the tissue. Concerning the biomedical application the sample is represented by the turbid media which is bio-tissues or biological liquids. Thus, the optical properties of tissues influence the quality of OCT images and on the appearance of the structure within these images making it possible to obtain morphological texture of the samples.

Taking into account aforesaid information it is possible to reconstruct the morphological structure of the sample in 2D and 3D by measuring the intensity of light backscattered at different depth position of the sample (Huang *et al.*, 1991; Welzel, 2001). Such imaging set up is usually called conventional OCT. There is also another type of OCT which is named Polarization-Sensitive Optical Coherence Tomography (PS-OCT). The latter is taking into account the polarized nature of radiation, thus, additional information about internal structure can be obtained from the specimens with birefringency, such as tendons (Bagnaninchi *et al.*, 2007; Ugryumova *et al.*, 2005) or collagen fibres (Brezinsky and Fujimoto, 2002; Pierce *et al.*, 2004; Matcher *et al.*, 2004).

OCT in addition with Doppler Effect allows also evaluating multi-dimensional velocity distribution of moving objects within the sample (Bonesi *et al.*, 2007; Veksler *et al.*, 2008). This is possible because of the appearance of Doppler Effect when the probing radiation is interacting with moving particles within the tissues. Such imaging technique is called Doppler OCT, detailed description of which will be presented in the next paragraph.

These three imaging modalities can be obtained from any TD-OCT, FD-OCT or SS-OCT system implementation (Choma *et al.*, 2003; Leitgeb *et al.*, 2003; Yasuno *et al.*, 2004; Wojtkowski *et al.*, 2004; Zhang and Chen, 2005).

The application of these OCT techniques allows achieving improvement of the quality of biomedical imaging in different areas, e.g. ophthalmology, tissue engineering (Bagnaninchi *et al.*, 2007; Veksler *et al.*, 2008) or cardiology. Detailed review of the major applications of OCT can be found in (Bouma and Tearney, 2002; Fercher *et al.*, 2003; Sampson, 2004; Tomlins and Wang, 2005; Brezinsky, 2006; Regar *et al.*, 2007).

5.2 Doppler OCT

Doppler OCT is a functional extension of OCT which can provide possibility of reconstruction of the internal structure of the sample with high resolution and at the same time to visualize micro-flows velocity distribution profiles of moving particles within the same sample. The measurement of the micro-flow velocity becomes possible with the usage of the Doppler Effect, the basic principle of which lies in the appearance of the frequency shift (so called Doppler shift) of the electromagnetic wave during the illuminating with radiation of the sample with moving particles within.

In vacuum Doppler shift for the electromagnetic (EM) waves can be represented as

$$f_D = \frac{\sqrt{1 - \frac{u^2}{c^2}}}{1 \mp \frac{u}{c}} f_0 = \frac{\sqrt{c^2 - u^2}}{c \mp u} f_0, \quad (5.17)$$

where c is the speed of light in vacuum; u is the relative velocity of the moving particle, evaluated along the propagation direction of the light and considered positive when the particle is approaching the observer; f_0 is the frequency of the radiation incident on the particle and f_D is the observed frequency of the EM radiation reflected by the particle – namely the Doppler frequency or the Doppler shift. The sign ‘–’ in the denominator indicates that the particle is approaching the observer, whilst ‘+’ indicates that the particle is receding from the observer.

For EM wave propagating in a media with refractive index n , Equation 5.17 will be the next

$$f_D = \frac{\sqrt{1 - \frac{u^2}{v^2}}}{1 \mp \frac{u}{v}} f_0 = \frac{\sqrt{v^2 - u^2}}{v \mp u} f_0, \quad (5.18)$$

where $v = nc$ is the speed of light in the media.

Eq. (5.17) and similarly Eq. (5.18) can be rewritten in terms of wavelength λ of the EM radiation taking into account the fact that $c = \lambda f = \text{const}$ as

$$\lambda_D = \frac{c \mp u}{\sqrt{c^2 - u^2}}, \quad (5.19)$$

where λ_0 is the wavelength of the incident radiation and λ_D is the wavelength of the radiation observed after the interaction with the moving particle.

With reference to a time-domain Doppler OCT system, in addition to the structural information, to retrieve data regarding velocity of flowing particles within the sample, estimation of the Doppler shift frequency superimposed at the probing radiation and stored on the acquired interferometric signal must be computed.

Each pixel associated to a spatial point within the sample composing a Doppler OCT image is generated by heterodyne detection of the two waves

$$E_t = E_1 \cos(kz + \omega_0 t + \varphi_1), \quad (5.20)$$

$$E_s = E_2 \cos(kz + \omega_D t + \varphi_2) \quad (5.21)$$

travelling in the reference arm and the sample arm of the interferometer respectively (Bouma and Tearney, 2002; Fercher *et al.*, 2003; Brezinski, 2006). Here $\omega_0 = 2\pi f_0 = 2\pi V_m/\lambda_0$ is the carrier frequency due to the scan movement; $kz = 2\pi ct/\lambda_0$ is the phase of the electric field; V_m is the velocity of the moving element in the scan system, generally linearly time-dependent; c is the speed of the light in the medium; λ_0 is the light source central wavelength; z is the scanning distance; t is the time; φ_i ($i = 1, 2$) represent the phase of the wave evaluated at $t = 0, z = 0$; $\omega_D = 2\pi f_D = 2\pi V_z/\lambda_0$ is the Doppler frequency shift evaluated at each depth point in the sample and V_z is the resolved velocity component parallel to the beam propagation direction. Both waves are mixed on the detector and, after eliminating DC components and filtering low-frequency components from the heterodyne detected signal, the observed electronic signal can be described by:

$$I_d(t) = I_0(t) \cos[(\omega_0 - \omega_D)t + \Delta\varphi(t)], \quad (5.22)$$

where $\Delta\varphi(t) = \varphi_1 - \varphi_2$ and $I_0(t)$ is the amplitude modulation of the interferometric signal due to sample heterogeneities and carries the structural information (morphology) of the sample.

The Doppler shift (see Figure 5.2) evaluated at every in-depth position is expressed by the difference $(\omega_0 - \omega_D)$, and includes velocities variations arising both from the reference arm (ω_0) and sample arm (ω_D).

Assuming $V_m = \text{const}$, *i.e.* constant velocity of the scanning element in the reference arm, the term ω_0 in Equation 5.22 can be neglected. Hence, only the Doppler shift variations due to the motion of the flow in the sample arm are considered. The Doppler shift can be evaluated by (Gill, 1965; Gjurchinovski, 2004)

$$\omega_D = (\mathbf{k}_s - \mathbf{k}_i) \mathbf{V}_D, \quad (5.23)$$

where $\mathbf{k}_s$ and $\mathbf{k}_i$ are the wave vectors of the backscattered and incident light respectively and $\mathbf{V}_D$ is the velocity vector of the moving constituents. With knowledge of the angle θ between $(\mathbf{k}_s - \mathbf{k}_i)$ and $\mathbf{V}_D$, measurement of ω_D allows the point flow velocity to be determined.

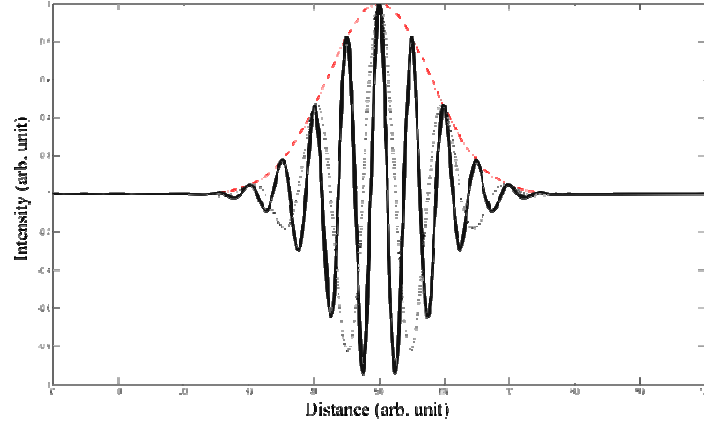


Figure 5.2. Effect of the interaction of the probing radiation with a moving particle on the cross-correlation interferometric signal. The red curve represents the envelope of the cross-correlation signal and doesn't change. The black curve represents the phase of the cross-correlation signal and is sensitive to changes in frequency due to the Doppler effect; the dotted curve represents a change in the phase of the signal due to the Doppler interaction between the probing radiation and a moving particle in the sample.

To detect the non-zero Doppler shift, the difference $(\mathbf{k}_s - \mathbf{k}_i)$ must have a non-vanishing component along a direction parallel to the velocity vector $\mathbf{V}_D$, this latter evaluated at the backscattering centre. Assuming $\mathbf{k}_s = -\mathbf{k}_i$ (single backscattering event coupled back into the fibre), the estimation of the flow velocity can be expressed via the Doppler formula (Bouma and Tearney, 2002; Fercher *et al.*, 2003; Brezinski, 2006)

$$|\vec{V}_D|_z = \frac{f_D \lambda_0}{2n \cos \alpha}, \quad (5.24)$$

where n is the refractive index of the sample, f_D is the centroid of the Doppler spectrum and the subscript z denote the fact that only the flow velocity component evaluated along the beam direction is detected.

If $f_D = \omega_D/2\pi \neq 0$, i.e. presence of moving scattering particles with velocity $\pm V_{D,z} \neq 0$, the Doppler frequency shift will be to the left or to the right of the modulation frequency $f_0 = \omega_0/2\pi$ introduced by the scanning device, depending on the sign of $V_{D,z}$. The difference between f_D and f_0 is proportional to the magnitude of the flow velocity component evaluated along the beam direction. The quantity $(\omega_0 - \omega_D)$, or, more particularly, ω_D , can then be numerically estimated following different strategies, including Fourier analysis applied on each A-scan separately and detection of phase changes between consecutive A-scans. The adoption of a particular strategy give rise to classifications of Doppler OCT technique in sub-classes, the most relevant of which are briefly analyzed in the following paragraphs.

5.2.1 Basic principles of Doppler OCT imaging

The most straightforward method to detect the Doppler frequency shift is spectral analysis using the Fourier transformation. The method is based on calculations of the *Short-Term Fast Fourier Transform* (ST-FFT) algorithm on each acquired A-scan, generating a *spectrogram* as outcome. (Chen *et al.*, 1997a; Chen *et al.*, 1997b; Brezinski, 2007). Details on theoretical and practical aspects of the ST-FFT algorithm can widely be found in literature (see for example Meyer, 1995). In its more general conception, the spectrogram is the result of calculating the frequency spectrum of windowed frames of a compound signal. It is a three-dimensional plot of the energy of the frequency content of a signal as it changes over time. With reference to a single A-scan, the implementation of ST-FFT for Doppler OCT generates a 2D plot in the time-frequency domain (abscissa and ordinate axis) with colour-coded spectrum amplitude. The graph represents the instantaneous frequency content of the signal, which is then converted into instantaneous velocity information at every depth inside the sample. For a time-domain OCT system, the knowledge of the time-dependent law that defines the position of the scan element in time allows to convert the

time scale of the spectrogram into distances, which represent the axial position (depth) of the measured element (voxel) inside the sample. The velocity information is obtained converting the instantaneous frequency Doppler shift.

The chosen parameters of the ST-FFT computation define the resolution of the time-frequency scales, influencing velocity sensitivity and spatial resolution in Doppler OCT. The definition of the window size is critical to the ST-FFT result and the performance of the velocity estimation. The relationship between frequency resolution δf and time resolution δt follows Heisenberg uncertainty

$$\delta f \delta t \geq \frac{1}{4\pi}. \quad (5.25)$$

Higher time resolution, *i.e.* higher spatial resolution, is obtained with narrow Fourier transform window but will result in lower frequency resolution, *i.e.* poor velocity resolution. The major limitation of this technique applied to Doppler OCT imaging is that this method depends on the FFT window time, which limits axial scanning speed and spatial resolution, as described in the following paragraph. For example, this can represent a serious limitation when measuring slow-moving blood flow in small vessels, which requires high velocity sensitivity at spatial high-resolution.

5.2.2 Sensitivity and resolution

The digital detection of the Doppler shift is obtained computing the spectrogram via ST-FFT algorithm on each acquired A-scan. Since the detection of the Doppler shift requires sampling of the interference fringe intensity over at least two samples per oscillation cycle, the minimum detectable Doppler frequency shift, Δf_D^{min} varies inversely with the FFT time-window size, N , and the sampling interval, T_s ,

$$\Delta f_D^{min} = \frac{1}{NT_s}, \quad (5.26)$$

where NT_s defines the window time interval. This introduces a trade-off between velocity sensitivity and scanning speed as well as spatial resolution (Kulkarni *et al.*, 1998; Zhao *et al.*, 2000). The sampling frequency $f_s = 1/T_s$ and the ST-FFT parameter N determine the velocity resolution, defined as the minimum resolvable velocity, V_D^{min} , which is directly proportional to the minimum detectable Doppler shift (Bouma and Tearney, 2000).

$$V_D^{min} = \frac{\lambda_0}{2n \cos \theta} \frac{1}{NT_s}. \quad (5.27)$$

Larger values of NT_s will increase the velocity sensitivity. However, the depth spatial resolution δz , is proportional to the ST-FFT time-window as follow:

$$\delta z = V_m NT_s, \quad (5.28)$$

where V_m is the velocity of the moving elements in the scanning device of the reference arm. Equations 5.27 and 5.28 define the velocity resolution and the spatial resolution of a Doppler OCT system respectively. These two quantities are coupled via the ST-FFT time-window term NT_s , increasing this value will give a better velocity sensitivity at the cost of sacrificing the spatial resolution. A reduction of V_m could improve δz , but implementing high-speed scan velocity must be taken in account in order to minimize motion related artefacts. High spatial resolution is obtained at the cost of sacrificing high velocity sensitivity. Furthermore, the precision of the estimated Doppler shift is also limited by the Doppler signal bandwidth, which is proportional to the optical source spectral width (Kulkarni *et al.*, 1998), which will result in a practical velocity resolution worse than the one estimated with Equation 5.27. For example, if a Doppler OCT image consisting of 100×100 pixels is acquired with a frame rate of 1 Hz, the acquisition time of each pixel is

0.1 ms, corresponding to a minimum resolvable Doppler shift of 10 KHz. This results in a velocity sensitivity of $25 \text{ mm}\cdot\text{s}^{-1}$ for a light source with central wavelength of 1300 nm at an angle of 80° to the direction of the flow.

The examples of the results of experiments on flow measurements with the help of Doppler OCT are presented on the Figures 5.3 and 5.4.

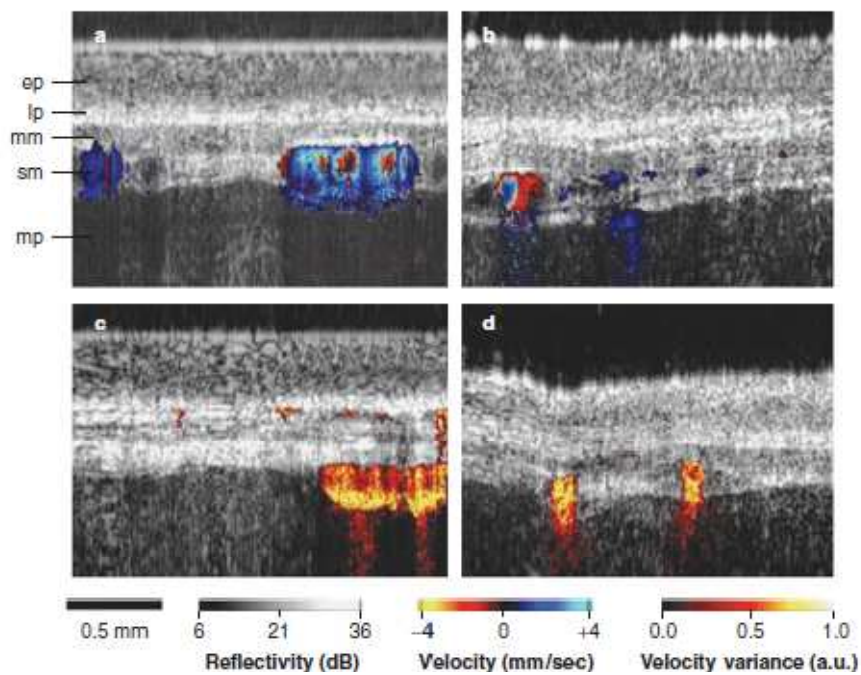
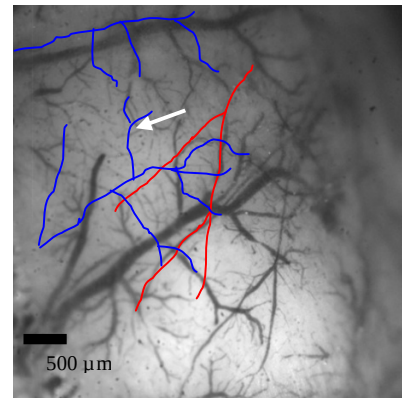
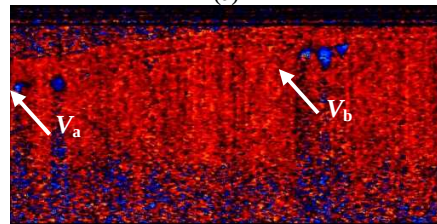


Figure 5.3. Human endoscopic D-OCT images of normal esophagus with the five layers indicated: epithelium (ep), lamina propria (lp), muscularis mucosa (mm), submucosa (sm) and muscularis propria (mp). (a and b) Color Doppler images showing vessels of microcirculation in the muscularis mucosa, submucosa and muscularis propria. The color rings in the larger vessels were caused by aliasing, due to the flow velocity exceeding the maximum detection range ($\pm 4 \text{ mm/s}$). (c and d) Velocity-variance images of the microcirculation in the lamina propria, muscularis mucosa, submucosa and muscularis propria. Few blood vessels were observed in the epithelium. The probe was in contact with the epithelial surface and the compression flattened the epithelium in (a) and (c) (Yang et al., 2005).



(a)



(b)

Figure 5.4. *In vivo* measurements of sub-cranial cerebral blood flow of a rat. Top-view photography of the rat blood vessels net (a). The arrow indicates the measured area. (b) DOCT tomogram (4 mm x 2 mm). The arrows indicate the presence and position of the blood vessels (V_a and V_b) (Bonesi et al., 2009).

5.3 Principles of Confocal Laser Scanning Microscopy (CLSM)

The technique of laser scanning confocal fluorescence microscopy nowadays has become an essential tool in biology and associated biomedical sciences, as well as in materials science due to attributes that are not readily available using other contrast modes with traditional optical microscopy (Pawley, 1995; Paddock, 1999; Diaspro, 2002; Matsumoto, 2002; Sheppard and Shotton, 1997; Muller, 2002; Hibbs, 2004; Conn, 1999; Corle and Kino, 1996; Wilson, 1990; Gu, 1996; Masters, 1996). The application of a wide array of new synthetic and naturally occurring fluorochromes has made it possible to identify cells and sub-microscopic cellular components with a high degree of specificity amid non-fluorescing material (Mason, 1999). In fact, the confocal microscope is often capable of

revealing the presence of a single molecule (Peterman *et al.*, 2004). Through the use of multiply-labeled specimens, different probes can simultaneously identify several target molecules simultaneously, both in fixed specimens and living cells and tissues (Goldman and Spector, 2005). Although both conventional and confocal microscopes cannot provide spatial resolution below the diffraction limit of specific specimen features, the detection of fluorescing molecules below such limits is readily achieved.

The basic concept of confocal microscopy was originally developed by Marvin Minsky in the mid-1950s (patented in 1961) (Minsky, 1961; Minsky, 1988). The aim of Minsky was to visualize neural networks in unstained preparations of brain tissue. The first commercial instruments appeared in 1987. During the 1990s, advances in optics and electronics afforded more stable and powerful lasers, high-efficiency scanning mirror units, high-throughput fiber optics, better thin film dielectric coatings, and detectors having reduced noise characteristics (Pawley, 1995). Modern confocal microscopes can be considered as completely integrated electronic systems where the optical microscope plays a central role in a configuration that consists of one or more electronic detectors, a computer (for image display, processing, output, and storage), and several laser systems combined with wavelength selection devices and a beam scanning assembly. In most cases, integration between the various components is so thorough that the entire confocal microscope is often collectively referred to as a digital or video imaging system capable of producing electronic images (Spring and Inoue, 1997). These microscopes are now being employed for routine investigations on molecules, cells, and living tissues that were not possible just a few years ago (Goldman and Spector, 2005).

5.3.1 Basic concepts of CLSM

Confocal microscopy offers several advantages over conventional widefield optical microscopy, including the ability to control depth of field, elimination or reduction of background information away from the focal plane (that leads to image degradation), and the capability to collect serial optical sections from thick specimens. The basic key to the

confocal approach is the use of spatial filtering techniques to eliminate out-of-focus light or glare in specimens whose thickness exceeds the immediate plane of focus.

The confocal principle in epi-fluorescence laser scanning microscope is diagrammatically presented in Figure 5.5. Coherent light emitted by the laser system (excitation source) passes through a pinhole aperture that is situated in a conjugate plane (confocal) with a scanning point on the specimen and a second pinhole aperture positioned in front of the detector (a photomultiplier tube). As the laser is reflected by a dichromatic mirror and scanned across the specimen in a defined focal plane, secondary fluorescence emitted from points on the specimen (in the same focal plane) pass back through the dichromatic mirror and are focused as a confocal point at the detector pinhole aperture.

The significant amount of fluorescence emission that occurs at points above and below the objective focal plane is not confocal with the pinhole (named Out-of- Focus Light Rays in Figure 5.5) and forms extended Airy disks in the aperture plane (Stelzer, 2000). Because only a small fraction of the out-of-focus fluorescence emission is delivered through the pinhole aperture, most of this extraneous light is not detected by the photomultiplier and does not contribute to the resulting image. The dichromatic mirror, barrier filter, and excitation filter perform similar functions to identical components in a widefield epi-fluorescence microscope (Rost, 1992). Refocusing the objective in a confocal microscope shifts the excitation and emission points on a specimen to a new plane that becomes confocal with the pinhole apertures of the light source and detector.

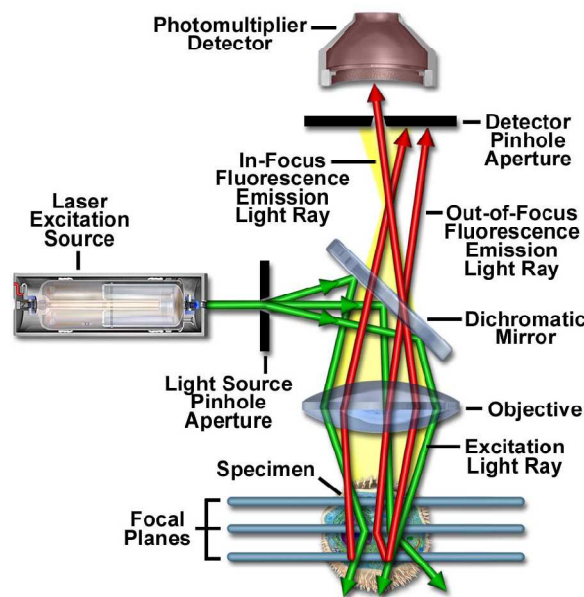


Figure 5.5. Schematic diagram of the optical pathway and principal components in a laser scanning confocal microscope.

Basic microscope optical system characteristics have remained fundamentally unchanged for many decades due to engineering restrictions on objective design, the static properties of most specimens, and the fact that resolution is governed by the wavelength of light. However, fluorescent probes that are employed to add contrast to biological specimens and, and other technologies associated with optical microscopy techniques, have improved significantly.

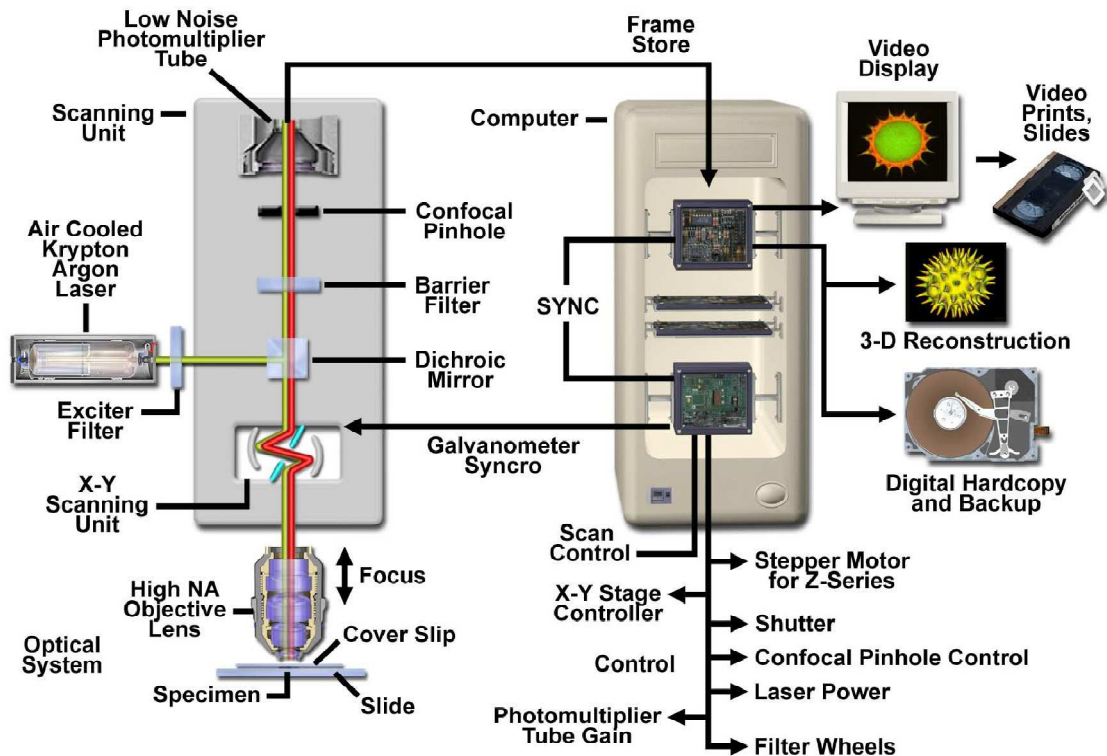


Figure 5.6. *Confocal microscope configuration and information flow schematic diagram.*

The explosive growth and development of the confocal approach is a direct result of a renaissance in optical microscopy that has been largely fueled by advances in modern optical and electronics technology. Among these are stable multi-wavelength laser systems that provide better coverage of the ultraviolet, visible, and near-infrared spectral regions, improved interference filters (including dichromatic mirrors, barrier, and excitation filters), sensitive low-noise wide band detectors, and far more powerful computers. The latter are now available with relatively low-cost memory arrays, image analysis software packages, high-resolution video displays, and high quality digital image printers. The flow of information through a modern confocal microscope is presented diagrammatically in Figure 5.6.

5.3.2 Advantages and Disadvantages of Confocal Microscopy

The primary advantage of laser scanning confocal microscopy is the ability to serially produce thin (0.5 to 1.5 micrometer) optical sections through fluorescent specimens that have a thickness ranging up to 50 micrometers or more (Sandison and Webb, 1994). The image series is collected by coordinating incremental changes in the microscope fine focus mechanism (using a stepper motor) with sequential image acquisition at each step. Image information is restricted to a well-defined plane, rather than being complicated by signals arising from remote locations in the specimen. Contrast and definition are dramatically improved over widefield techniques due to the reduction in background fluorescence and improved signal-to-noise (see Figure 5.7) (Sandison and Webb, 1994). Furthermore, optical sectioning eliminates artefacts that occur during physical sectioning and fluorescent staining of tissue specimens for traditional forms of microscopy. The non-invasive confocal optical sectioning technique enables the examination of both living and fixed specimens under a variety of conditions with enhanced clarity.

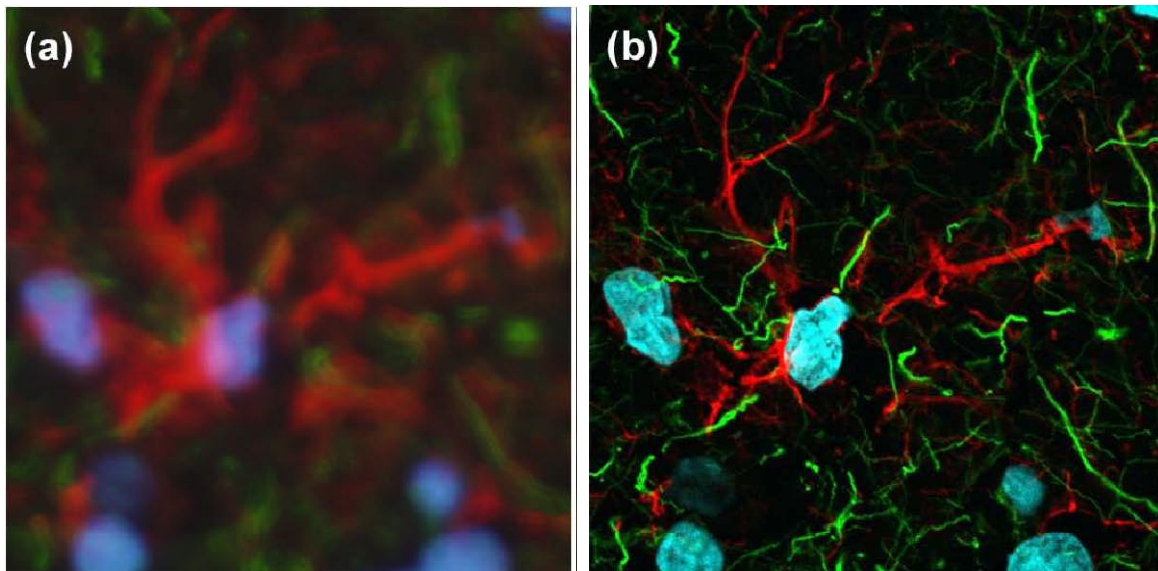


Figure 5.7. Comparison of widefield (a) and laser scanning confocal fluorescence microscopy images (b).

Advances in confocal microscopy have made possible multi-dimensional views of living cells and tissues that include image information in the x, y, and z dimensions as a function of time and presented in multiple colors (using two or more fluorophores).

Disadvantages of confocal microscopy are limited primarily to the limited number of excitation wavelengths available with common lasers (referred to as laser lines), which occur over very narrow bands and are expensive to produce in the ultraviolet region. In contrast, conventional widefield microscopes use mercury or xenon based arc-discharge lamps to provide a full range of excitation wavelengths in the ultraviolet, visible, and near-infrared spectral regions. Another downside is the harmful nature (Ashkin *et al.*, 1987) of high-intensity laser irradiation to living cells and tissues, an issue that has recently been addressed by multiphoton and Nipkow disk confocal imaging. Finally, the high cost of purchasing and operating multi-user confocal microscope systems (DeMaggio, 2002), which can range up to an order of magnitude higher than comparable widefield microscopes, often limits their implementation in smaller laboratories. This problem can be easily overcome by cost-shared microscope systems that service one or more departments in a core facility. The recent introduction of personal confocal systems has competitively driven down the price of low-end confocal microscopes and increased the number of individual users.

5.4 Summary

In this chapter the basic principle of low-coherence optical techniques were presented. These methods are of high priority because they can provide rather cheap and convenient non-invasive optical diagnostics of biotissues. Combining the existing OCT and CLSM methods with the results of our MC modeling the parameters of the set-ups were optimized and the results of experiments will be presented in the next chapters.

Chapter 6. Validation, improvement and development of Monte Carlo method

This chapter includes the results of validation and verification of the MC code described in previous chapter. As a typical set-up fibre optics probe was considered. Existing code was improved with including of the coherence properties of light within the code. Additionally PS-OCT was simulated with the help of MC. Then, based on the results of simulations OCT parameters were optimized (will be described in the next chapter).

6.1 Validation: Impact of probe geometry on the detecting field of view

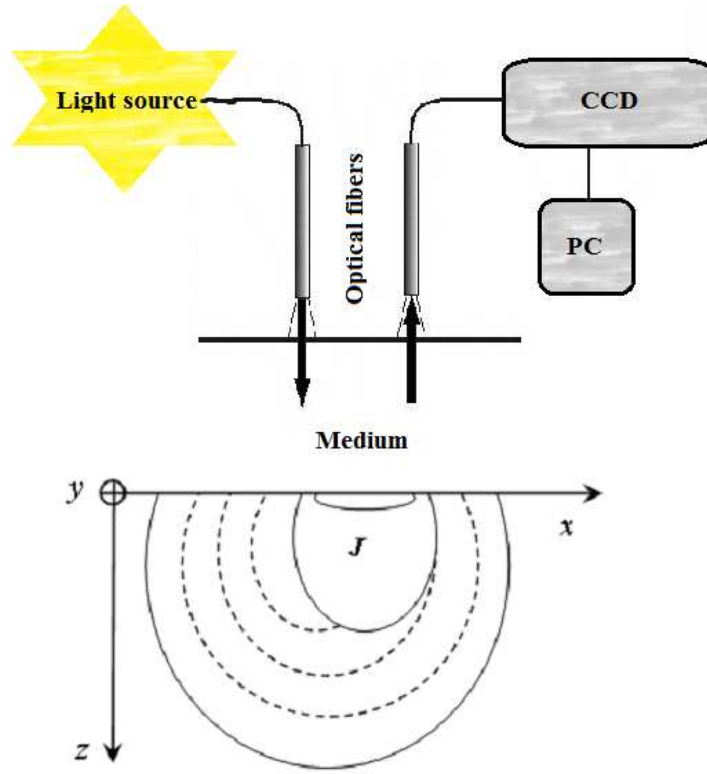


Figure 6.1. Schematic representation of the experimental set-up, and definition of sampling volume distribution. Here, J is the spatial distribution of the effective optical paths, e.g. sampling volume.

Due to the multiple scattering incident radiation is widely distributed within bio-tissues. Whereas the detected signal is spatially localized in the area called sampling volume. The peculiarities of this spatial localization of the detected signal are determined by the geometrical and optical properties of the medium as well as by the probe geometry. Analysis of sampling volume is extremely useful for quantitative interpretation of the experimental results, analysis of the detected regions within the medium covered by the sounding radiation, and finally for prediction of optimal probe geometry of particular experimental systems applied for day-to-day clinical diagnostics.

Three source-detector geometries typically used in the clinical applications, including optical fibre probe, CCD camera and combination of optical fibre and CCD was considered (Fig.6.1). The source and detector are located on the surface of the medium. The source is represented by a collimated laser beam, or a laser beam guided by an optical fibre, or by a flash lamp. The direct measurements of the sampling volume cannot be obtained experimentally, but can be estimated by computational modelling. To describe the propagation of laser radiation within the complex random media the Monte Carlo (MC) method is used (Meglinski and Matcher, 2001) to predict sampling volumes distribution within the human skin. In case of this complex inhomogeneous multilayer medium, where the optical properties differ for each layer, it is possible to consider the partial components of sampling volume for each layer.

The multilayered model of skin which consists of 7 layers of cells was considered in the research. Each layer has its own optical properties, which are presented in the next table.

Table 6.1

	1	2	3	4	5	6	7
μ_s, mm^{-1}	80	35	30	25	20	30	5
μ_a, mm^{-1}	0.1	0.15	0.068	0.095	0.073	0.118	0.068
g	0.8	0.8	0.8	0.9	0.76	0.95	0.75
n	1.5	1.34	1.39	1.4	1.39	1.38	1.44

The sampling volume was simulated for various probe geometry (Fig.6.2).

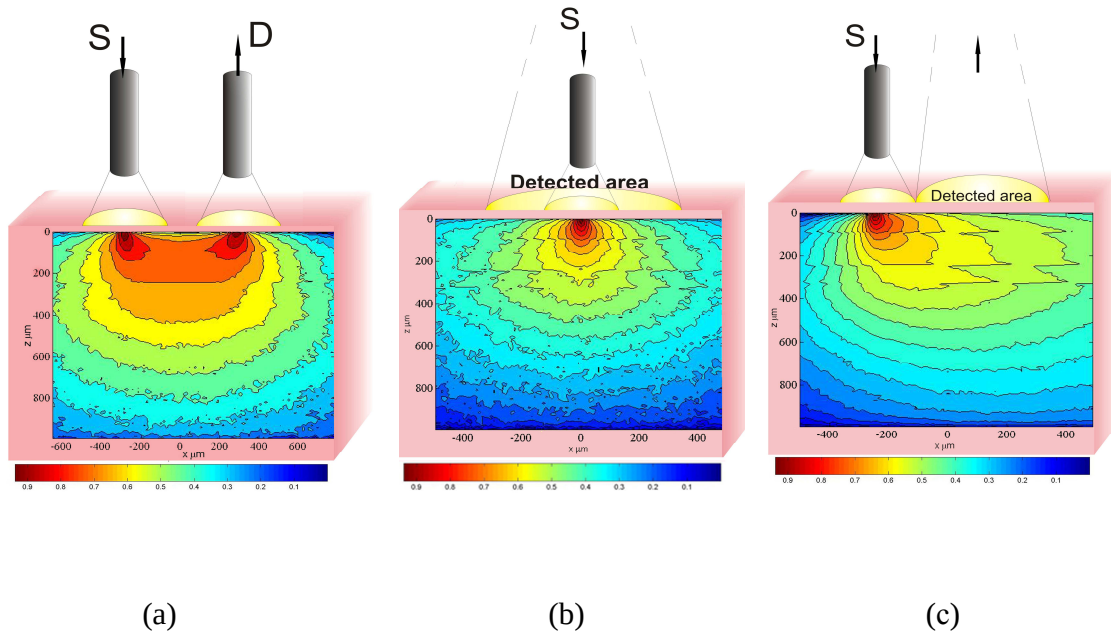


Figure 6.2 Sampling volume $J(x,z)$ in a multilayered medium for configurations with: (a) optical fibres; (b) CCD camera; (c) combination of optical fibre and CCD.

The results of the simulation clearly show that probe with separated source-detector field of view significantly improve the localization of detected signal within the medium.

6.2 Improvement: New computational model for simulation of polarized light propagation within biological tissues

New computational method that is able to consider all the scattering orders within a detected OCT signal was developed. The method is based on the iterative procedure of the solution of Bethe-Salpeter equation (Kuzmin *et al.*, 2005). The new approach is able adequately account for the ballistic component of the detected OCT signal including the contribution of the rear interfaces within OCT signal.

In the context of the Monte Carlo method the non-coherent component of intensity can be presented as:

$$I(\mu_b, \mu_i) = \sum_{n < n_{sc}} I_n(\mu_b, \mu_i), \quad (6.1)$$

where $I_n(\mu_b, \mu_i)$ is the score of trajectories which contain n acts of scattering as an average on the sample N_{ph} of descending photons

$$I(\mu_b, \mu_i) = \frac{1}{N_{ph}} \sum_{i=1}^{N_{ph}} W_n^{(i)} \exp(-\mu_b z_n^{(i)}). \quad (6.2)$$

$W_n^{(i)}$ and $z_n^{(i)}$ represents statistical weight and distance to the boundary of the medium of photon scattered n times. Statistical weight $W_n^{(i)}$ is determined by the integrals $\int d\mathbf{r}_1 \dots d\mathbf{r}_n \prod_{j=1}^{n-1} \Lambda(\mathbf{r}_{j+1} - \mathbf{r}_j) p(\mathbf{q}_j)$, where $\Lambda(\mathbf{r}_1 - \mathbf{r}_2) = \exp\left(-\frac{|\mathbf{r}_1 - \mathbf{r}_2|}{l}\right) |\mathbf{r}_1 - \mathbf{r}_2|^{-1}$, and $\mathbf{q}_j = \mathbf{k}_j - \mathbf{k}_{j-1}$.

Obtained integral is calculated as statistical mean on the sample of values ξ_j defined with the random number generator. Phase function depends only on the angle of scattering θ_j : $p(\mathbf{q}_j) = p(\cos \theta_j)$.

The interferential component of back scattering is calculated similarly in case of small scattering angle θ_s . Each term of a series we should multiply by the phase multiplier

$$S_n^{(i)} = \cos \left[(\mathbf{k}_b + \mathbf{k}_i)_\perp (\mathbf{R}_n^{(i)} - \mathbf{R}_1^{(i)})_\perp \right] \approx \exp[k(x_n - x_1)\theta_s]. \quad (6.3)$$

The field inside the medium can be represented as a sum of the incident wave and the wave reflected from the rear boundary interface of the medium:

$$\langle E_s(\mathbf{r}, t) \rangle = E_s^{(in)}(\mathbf{r}, t) + E_s^{(out)}(\mathbf{r}, t). \quad (6.4)$$

The main difference of new developed approach compare to the conventional OCT modelling is that an additional wave describing ballistic component represented the reflection at the medium boundary (-ies) was taken into account.

The above-mentioned technique was employed to calculate the OCT signal at the position of the rear boundary for a slab of the thickness $d = 200 \mu m$. Other optical parameters of the medium were: refractive index 1.4, scattering anisotropy 0.9. Fig. 6.3 presents the comparison of OCT signals obtained with conventional model and model with additional wave.

Figure 6.3 demonstrates that the results calculated with both methods do not coincide for the range of optical densities that correspond to the intermediate scattering regime.

The ratio of the signals calculated with the suggested method and the conventional method is presented in Figure 6.4. As Fig. 6.4 suggests, for the small optical densities ($OD = \mu_s d$),

$OD < 0.3$, and big OD, $OD > 4$, the difference between the two methods is not so significant.

However, for the intermediate case the ratio increases that indicates that for ODs in the range of 0.3–4 there may be a pronounced numerical difference in the different approaches of estimating the contribution from the rear boundary.

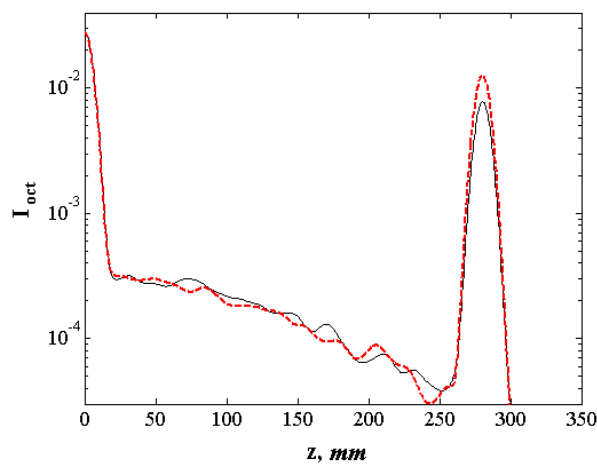


Figure 6.3. Intensities of the OCT signals calculated with the suggested (red line) method and the conventional method (black line).

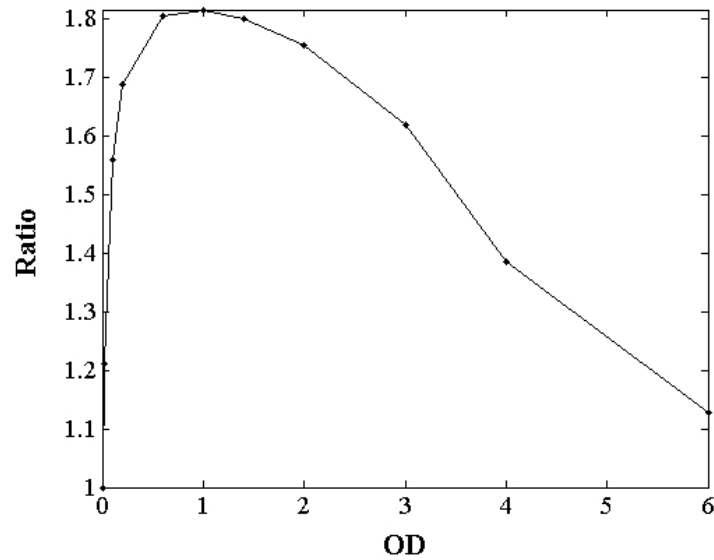


Figure 6.4. The ratio of the intensities of the OCT signals calculated with the suggested method, where two waves are considered, and the conventional method, where only one wave is regarded, as a function of the optical density OD of the slab.

6.3 Development: Simulation of polarization-sensitive optical coherence tomography images by a Monte Carlo method

Typically, within the MC algorithms the state of the polarization and its evolution during the propagation is described in framework of Stokes–Mueller or Jones formalism (Bartel and Hielscher, 2007; Wang and Wang, 2002; Ramella-Roman *et al.*, 2005). New MC technique was introduced, that is able to imitate two-dimensional (2D) polarization-sensitive OCT images of complex multilayered turbid scattering media avoiding implementation of Stokes–Mueller and/or Jones formalism.

6.3.1 Monte Carlo method in case of polarization

Following this let's consider propagation of photon packets along each possible trajectory within the medium. The free photon path between two successive scattering events is governed by Poisson distribution $f(l_i) = \mu_s \exp(-\mu_s l_i)$, and defined as $l_i = \mu_s^{-1} \ln \xi$, where ξ is the probability that the mean free path l is no less than l_i , arbitrary ξ value is chosen in the $[0,1]$ interval using a random number generator, and μ_s is the scattering coefficient. A new direction of the photon packet after each scattering event is determined by the Henyey–Greenstein scattering phase function (Wang *et al.*, 1995), originally developed to approximate Mie scattering of light from particles with size comparable with the wavelength of the incident light. This expression is characterized by a so-called anisotropy factor g , which is equal to the mean cosine of the scattering angle θ $g = \overline{\cos\theta}$ ($g \in [0,1]$, with $g=0$ corresponding to the isotropic scattering). A consideration of light scattering within an absorbing medium $\mu_s l \neq 1$ requires a proportional reduction of the statistical weight W of each photon packet according its trajectory (Churmakov *et al.*, 2002): $W = W_0 \exp(-\sum_{i=1}^N \mu_a l_i)$, where W_0 is the initial photon packet weight, μ_a is the absorption coefficient of the medium, and N is the number of experienced scattering events. The photon packets fitting the given detection criteria (Schmitt and Ben-Letaief, 1996), including size, numerical aperture, and detector position are taken into account. Completing the tracing of the photon trajectories for a large number of photons N_{ph} (typically $N_{ph} \sim 10^7$), the OCT signal is calculated as a convolution of distribution of the detected photons over their optical path lengths $L = \sum_{i=1}^N l_i$ with the envelope of coherence function:

$$I(z) = 2 \sum_{j=1}^{N_{ph}} \sqrt{W_r W_s L_j} \exp \left[- \left(\frac{z - L_j}{l_{coh}} \right)^2 \right], \quad (6.5)$$

where W_r and W_s are the total weights of the detected photon packets from the reference arm and scattering medium, respectively; l_{coh} is the coherence length of probing laser radiation; and z is the depth.

Using a combination of the MC technique and the iteration procedure of the solution of Bethe–Salpeter equation (Meglinski *et al.*, 2005), it has been shown that simulation of the optical path of a photon packet undergoing N scattering events directly corresponds to the N th order ladder diagram contribution. In this correspondence we generalized the above mentioned MC technique for the direct simulation evolution of the polarization vector for each photon packet. The polarization is described in terms of the polarization vector $\vec{P}$ undergoing a sequence of transformations after each scattering event (Ishimaru, 1978), and all the photon packets are weighted in accordance with the polarization state. In Rayleigh and Rayleigh–Gans approximations the polarization vector of the scattered wave $\vec{P}_i$ is transformed upon the i -th scattering such that (Ishimaru, 1978)

$$\vec{P}_i = -\vec{e}_i[\vec{e}_i \cdot \vec{P}_{i-1}] = [\hat{I} - \vec{e}_i \otimes \vec{e}_i] \vec{P}_{i-1}, \quad (6.6)$$

where $\vec{e}_i$ is the unit vector aligned along the trajectory element of a photon packet after the i -th scattering event. Thus, for simulating the electromagnetic field transfer we trace the transformation of the polarization vector of incident field $\mathbf{P}_0$ under the action of the chain of diadic operators:

$$\mathbf{P} = (\hat{I} - k^{-2} \mathbf{k}_s \otimes \mathbf{k}_s) \prod_{i=2}^N (\hat{I} - k^{-2} \mathbf{k}_{jj-1} \otimes \mathbf{k}_{jj-1}) \mathbf{P}_0. \quad (6.7)$$

The final stage of the parallel and perpendicular components of vector $\mathbf{P}$ determines the polarized and depolarized components of scattering radiation. In such a manner, we consider the propagation of polarized and depolarized components of the electromagnetic field along the trajectory defined for the intensity propagation (i.e., by scalar approach). However, and it should be pointed out here, based on the optical theorem (Carney *et al.*, 1997) the scalar approach gives (Kuzmin and Aksenova, 2003; Kuzmin *et al.*, 1994)

$$k_0^4 \int \tilde{G}(\vec{k}_i, \vec{k}_s) d\Omega_s = \frac{1}{l}, \quad (6.8)$$

whereas for the electromagnetic field in the limit of weak scattering (Kuzmin and Aksenova, 2003; Kuzmin *et al.*, 1994).

$$k_0^4 \int \tilde{G}(\vec{k}_i, \vec{k}_s) d\Omega_s = \frac{1}{l} \frac{2}{1 + \overline{\cos^2 \theta}}. \quad (6.9)$$

Here, $\tilde{G}(\vec{k}_i, \vec{k}_s)$ is the scattering phase function; $\vec{k}_i$ and $\vec{k}_s$ are the wave vectors for incident and scattered of complex-conjugated fields, respectively; and $k_0 = 2\pi/\lambda$, where λ is the wavelength. Therefore, the extra multiplicative factor

$$\Gamma = \frac{2}{1 + \overline{\cos^2 \theta}}. \quad (6.10)$$

should be taken into account at every scattering event. Finally, the expressions for the intensities of the co-polarized $I_{\parallel}$ and cross-polarized $I_{\perp}$ components valid for Henyey–Greenstein phase function are

$$\begin{aligned} I_{\parallel}(\chi) &= \sum_{j=1}^{N_{ph}} W_j \Gamma^{N_j} P_{\parallel j}^2, \\ I_{\perp}(\chi) &= \sum_{j=1}^{N_{ph}} W_j \Gamma^{N_j} P_{\perp j}^2, \end{aligned} \quad (6.11)$$

where N is the number of scattering acts for the j th photon packet and χ denotes the dependence on any variable of interest such as depth, time, number of scattering events, etc.

This MC algorithm has been validated against an accepted analytic solution for a semi-infinite medium, and cross validated for slab geometry with an absorbing inclusion. The results are compared with the exact solutions for pointlike Rayleigh scattering particles. For normal incidence and exact backscattering detection the generalized Milne solution for the electromagnetic field gives the ratio of polarized and depolarized intensities $I_{\parallel}/I_{\perp} = 1.92$ (Kuzmin and Aksenova, 2003). Numerical stimulation suggests that $I_{\parallel}/I_{\perp} = 1.93$ with all significant digits. The generalized Milne solution gives the depolarization ratio $DP = I_{\parallel} - \frac{I_{\perp}}{I_{\parallel}} + I_{\perp} = 0.31$ (Kuzmin and Aksenova, 2003), while the results of our simulation suggest $DP = 0.326$.

6.3.2 Results of the modelling and discussion

The skin model with six non-planar layers are shown in Fig.5.5. The optical properties of the model are presented in the Table 6.2.

Table 6.2

	1	2	3	4	5	6
μ_s, mm^{-1}	35	5	10	7	12	30
μ_a, mm^{-1}	0.02	0.015	0.02	0.1	0.7	0.2
g	0.9	0.95	0.85	0.9	0.87	0.95
n	1.54	1.34	1.4	1.39	1.4	1.39

The source-detector parameters are: wavelength $\lambda=1300\text{ nm}$, $l_{coh}=15\text{ }\mu m$, and numerical aperture 0.2. To simulate OCT images the sequential A-scans are simulated with the defined $20\text{ }\mu m$ transversal scanning step.

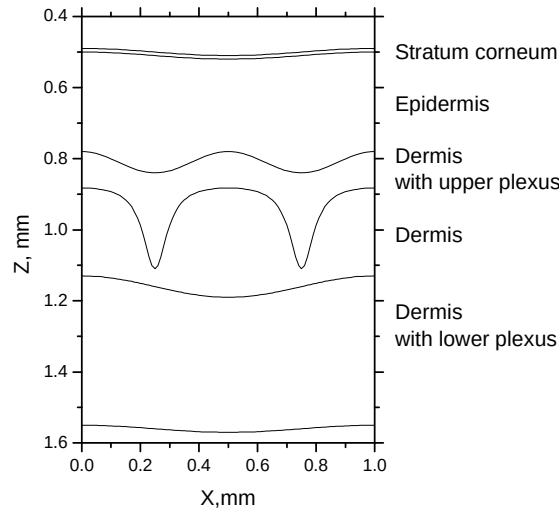


Figure 6.5. Schematic presentation of skin model.

The results of simulation of 2D OCT and polarization-sensitive OCT images for both co-polarized and cross-polarized modes are presented on Fig.6.6 in comparison with experimentally obtained images. The OCT image obtained in co-polarized mode (see Fig.6.6-b) is similar to the OCT image obtained for the convention OCT (Fig.6.6-a), whereas the intensity of the image obtained in cross-polarized mode (see Fig.6.6-c) is much lower compared with the co-polarized mode (Fig.6.6-b). This is due to the origin of the OCT signal formed mostly by so-called “snake” or low-scattering-order photons. Therefore, contribution of the photon packets depolarized owing to multiple scattering in the formation of the final OCT image is relatively small in upper layers and significantly rises for deep layers.

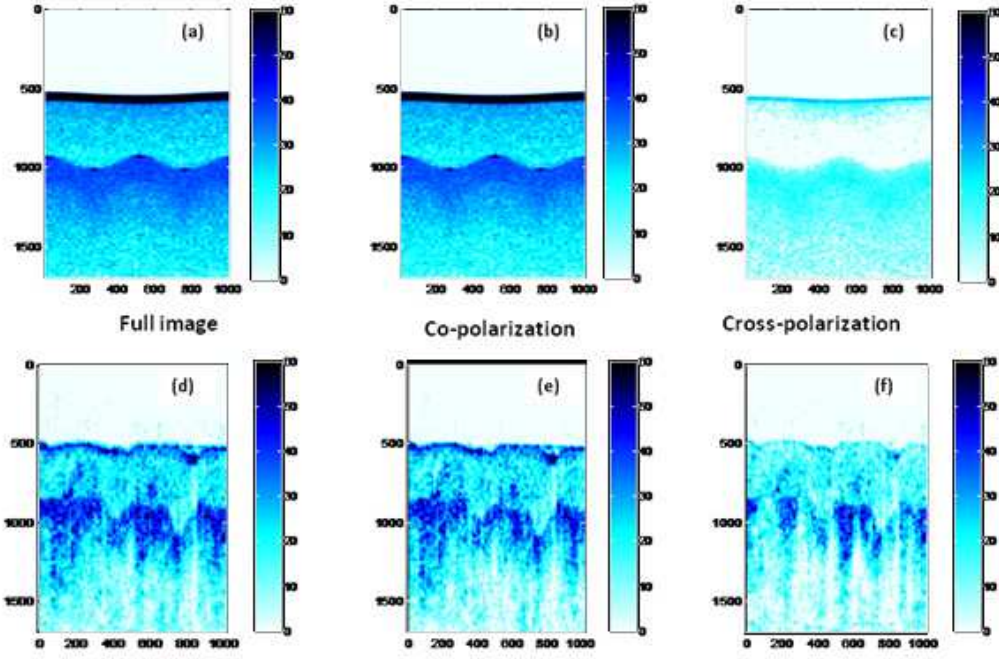


Figure 6.6. Comparison of simulated (a)-(c) and experimental (d)-(f) PS-OCT images.

Thus, based on the generalization of iterative procedure of the solution of Bethe–Salpeter equation, a new vector-based MC approach has been developed. The performances of the developed method are shown both for conventional and polarization-sensitive OCT modalities. The current approach allows considerably simplified simulation of polarized low-coherent optical radiation propagation within turbid scattering media avoiding implementation of Stokes–Mueller and/or Jones formalism, with the substantial reducing of the computational resources.

In a similar manner this approach can be applied for circular polarization allowing one to avoid a cumbersome calculation of the Mueller matrix. The technique can provide detailed information on polarization propagation within/outside the medium, scoring various physical quantities simultaneously. The technique will find a number of straightforward

applications related to the non-invasive diagnostics of various disperse random media, including biological tissues, polymers, liquid crystals, and others.

6.4 Development: Image reconstruction of the location of macro-inhomogeneity in random turbid medium by using artificial neural networks

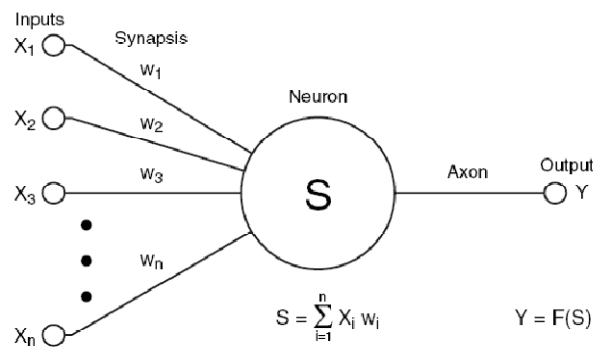


Figure 6.7. Schematic presentation of the artificial neuron.

In framework of optical tomography and related problems it is required to solve so-called inverse problem. This includes reconstruction of spatial positioning of the absorbing and/or scattering macro-inhomogeneities distribution within the medium by the characteristics of scattered radiation. This distribution depends on the distance between photo-detector and the entrance point of laser beam, and is determined by the scattering properties of the biological tissue. To describe the propagation of laser radiation within the complex random media conventionally statistical computational MC method, which combines the statistical weight scheme and effective optical photon paths simulation, was used. It is assumed that inhomogeneity located in subsurface area of the medium changes the intensity distribution

of the back-scattered radiation. This makes it possible to apply artificial neural networks (ANN) for finding the dimensions and locating of the inhomogeneity.

ANN consists with artificial neurons (Fig.6.7), connected together with synapses. The network processes input data that creates the complex of output signals. At present ANN technique is widely used to solve inverse problems in different biomedical applications (Frake *et al.*, 1998; Wallace *et al.*, 2000; Acton and Newberg, 2006).

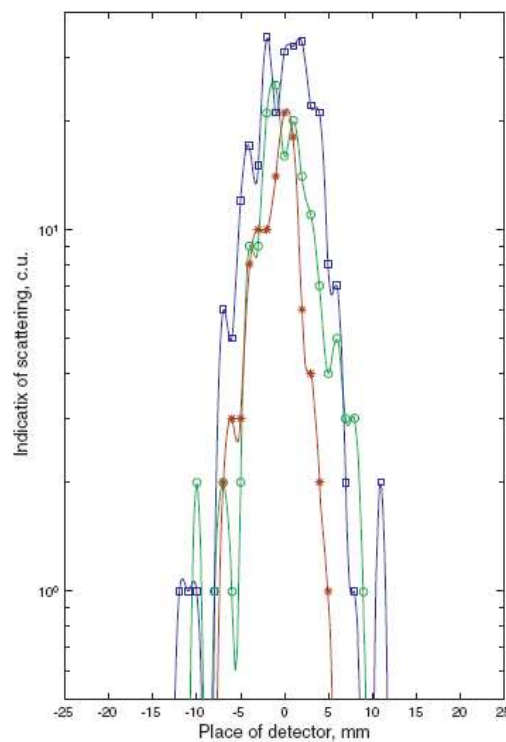


Figure 6.8. Intensity distribution of scattering at the surface of scattering medium (number of particles per unit of volume 10%); (*) in the absence of absorbing inhomogeneity in the topical layer of medium; (○) in the presence of absorbing inhomogeneities located in the left half-plane of the medium on the depth of 3–5 mm and (□) on the depth of 5 – 7 mm, the dimensions of the heterogeneity: 3×3 mm.

Fig. 6.8 represents the result of simulation of the intensity distribution at the surface of the modelling medium for different depth location of the inhomogeneity and with its absence.

These results are used as the training inputs for ANN that consisted with 20 neurons in the input layer, 5 neurons in hidden layer and 16 in output layer.

The image reconstruction of the absorbing inhomogeneity (Fig. 6.9) was restored by using the modelling intensities distributions presented in Fig. 6.8. It is clearly seen that when the object is located near the surface (see Fig. 6.9(a)) the ANN, trained by the results of MC model, restores the image of the inhomogeneity sufficiently well. The image of inhomogeneity becomes more diffuse and the ANN error substantially grows when the inhomogeneity gravitates deeper in the medium 7 mm (see Fig. 6.9(b)).

The presented results show the potential opportunities of use of ANN to solve the inverse problem. This is especially of high priority because of new diagnostics/treatment developing that is based on the applying gold nanoparticles for labelling cancer cells.

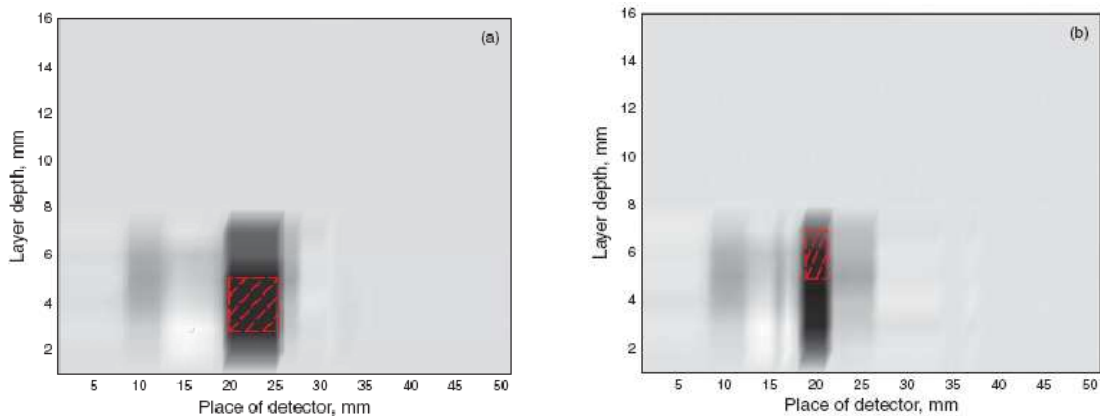


Figure 6.9. Locating of the inhomogeneity in the medium. Red dotted-line represents actual position of the inhomogeneity within the medium at the depth 3 – 5 mm (a), and 5 – 7 mm (b).

The proposed technique of image reconstruction has got further development in the works on dyes recognition of Ms. Ewa Moczko, see for example, Moczko *et al.*, 2009.

Chapter 7. Application and improvement of low-coherence methods of tissue diagnostics

7.1 Application of Optical Coherence Tomography for imaging of scaffold structure and micro-flows characterization

The conventional OCT was applied for the purpose of scaffold structure imaging. Three-dimensional porous scaffolds are used for the directional growth of the cells and for the supplying them with nutrients in tissue engineering. At the same time Doppler OCT was applied for the measurements of spatial fluid flow velocity distribution within this complex scaffold structure. Doppler OCT system is based on the typical fibre-optic Michelson configuration with 1x2 and 2x2 fibre couplers (Fig. 7.1).

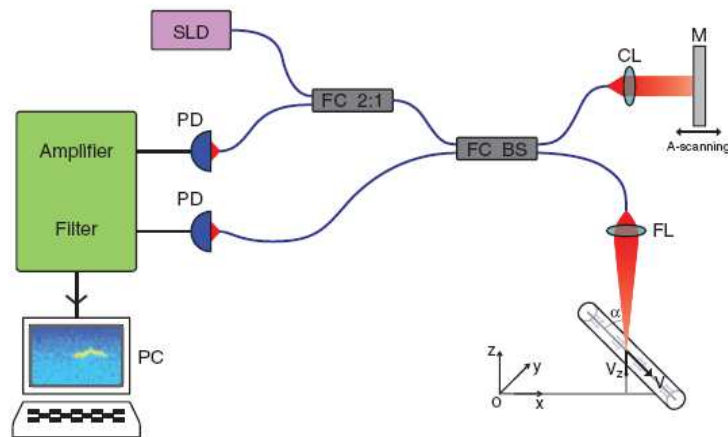
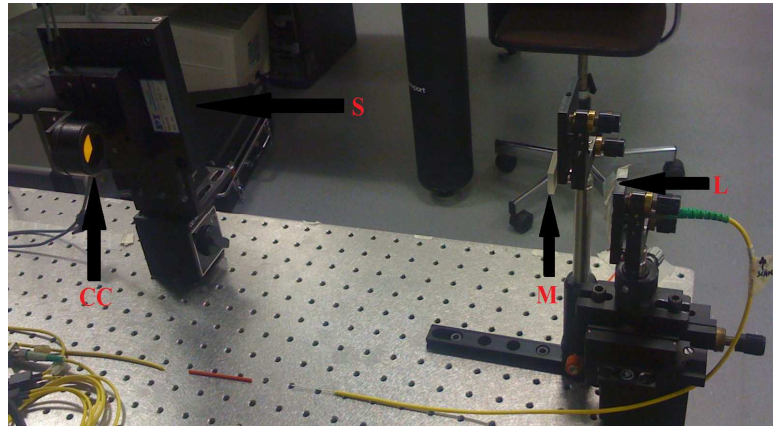
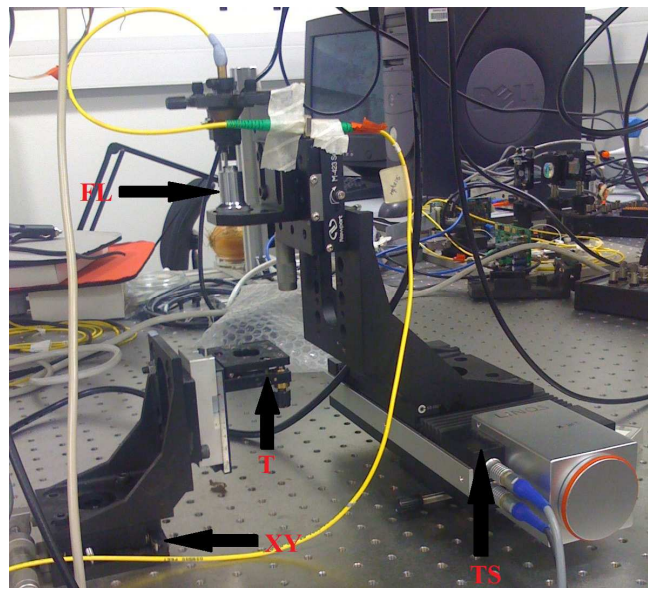


Figure 7.1. Schematic diagram of the experimental set-up. SLD – super-luminescence laser diode; FC – fibre coupler with splitter; CL – collimating lens; FL – focusing lens; M – reference arm scan mirror; PD – dual balanced photo-detector; PC – personal computer.



(a)



(b)

Figure 7.2. Experimental set-up. (a)-reference arm. L – collimating grin lens; CC – retroreflecting corner cube mounted on linear translation stage; S is the linear translation stage; M is the reflecting mirror. The motor (S) is connected to its driver and governed by a sawtooth signal generated by an external function generator.; (b) – sample arm. XY is the xy manual translational stage; T is the angular tilter; FL is the microscope objective (focusing lens); TS is the linear translational stage. The linear translational stage is controlled by its driver, interfaced with the PC.

The scaffolds, used in research, have been constructed from polysaccharidic material, called chitosan (Bagnaninchi *et al.*, 2007). 2% chitosan solution is prepared by dissolving chitosan flakes (95% deacylated, Sigma) in 1% v/v glacial acetic acid. The resulting solution is cast in several molds (1.5 mm inner diameter) which are frozen overnight and subsequently freeze dried. The resulting scaffolds are rehydrated in a gradient of ethanol (100%, 70%, 50%) and stored in Phosphate Buffered Saline (PBS). I used *Intralipid* (Sigma-Aldrich, 10%) for this experiment as a modelling liquid. *Intralipid* was set in motion by a syringe pump (Harward, mod. 975) allowing constant input volume flow rate under laminar regime (16.167 mm/s). *Intralipid* was diluted in distilled water to obtain an overall stock water solution at a concentration of 1% in volume. The optical properties of the solution agree well with the optical characteristics of nourishing liquids typically used in tissue engineering.

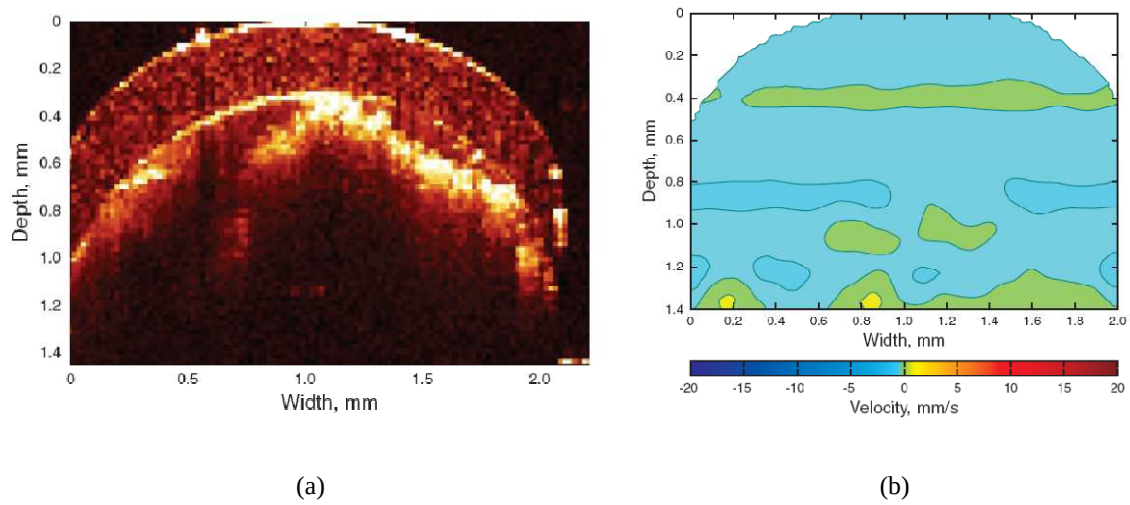


Figure 7.3. (a) - Image (2x2 mm) of the internal structure of scaffold obtained using the OCT; (b) - Cross-sectional flow velocity distribution of non-filtered 1% water solution of *Intralipid* through the scaffold. The input flow 16.167 mm/s.

Using the OCT system presented above (see Fig. 7.2) the structural image of the scaffold was obtained (Fig. 7.3(a)). The main peculiarities of the internal structure are clearly seeing however the porous nature of the sample is unclear. Fig. 7.3(b) demonstrates the flow velocity distribution within the scaffold obtained with Doppler OCT. The results of Doppler OCT measurements show that some fluctuations of velocity are observed, but the average velocity tends to zero, i.e. there is no flow present through the scaffold structure.

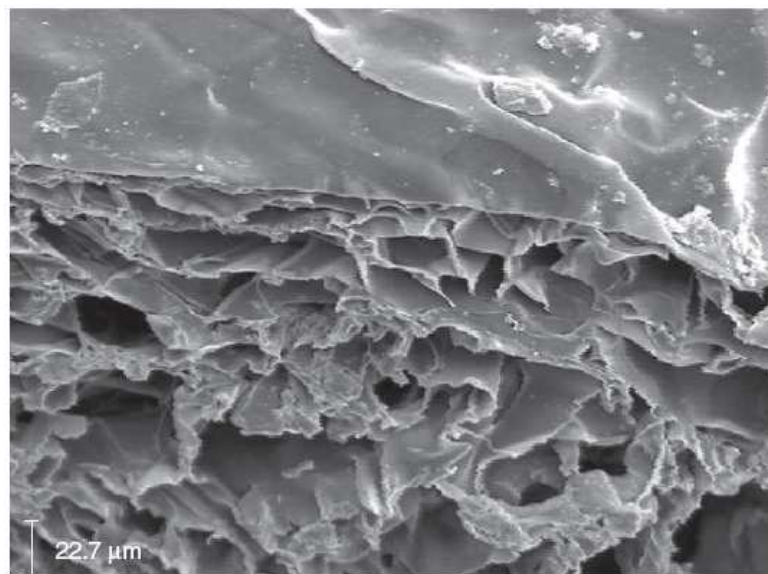


Figure 7.4. Microstructure of scaffold obtained with ISI ABT-55 low-vacuum scanning electron microscope.
The spatial resolution – 22.7 μm.

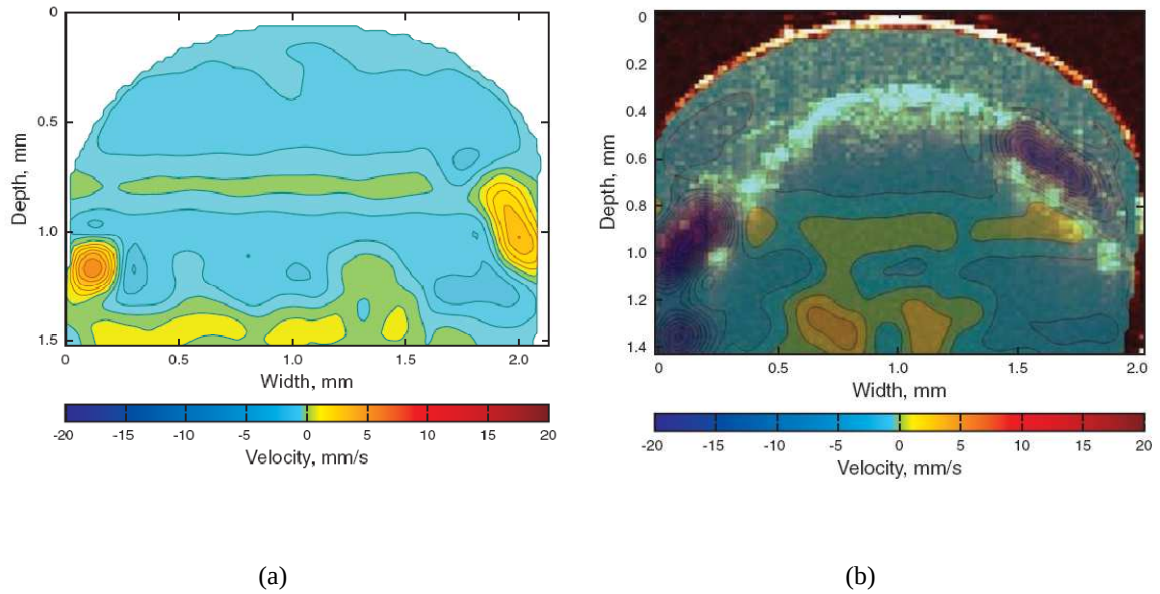


Figure 7.5. (a) - Cross-sectional flow velocity distribution of filtered water solution of Intralipid through the scaffold. The input flow 16.167 mm /s; (b) - Structural image (2×2 mm) of scaffold overlapped with the velocity flow distribution.

Assuming the possibility that large lipid particles in the solution can stack the pores within the scaffold we used scanning electron microscope (ISI ABT-55) to evaluate the actual pores size, as the OCT doesn't allow estimating the diameter of pores. The electron micrograph of the scaffold is presented on Fig. 7.4.

The average size of the pores appeared to be in a range of 20 – 40 μm with the effective diameter about 30 μm . *Intralipid* solution was filtered with a filter paper to avoid presence of the large lipid particles that can block the flow through the pores. The experiment was further repeated with a filtered water solution of *Intralipid*. The obtained results presented on Fig. 7.5(a) demonstrate distinctive differences in the velocity distribution in comparison with the results of non-filtered *Intralipid* flow measurements presented on Fig. 7.5(b).

It is seeing that the distribution of the flow of filtered *Intralipid* is not uniform and there are areas with higher velocity (up to 5 mm/s), compared to the overall background velocity. Fig. 7.5(b) shows the structural image overlapped with the representation of the velocity flow distribution. Thus, these results make it possible to evaluate the shear stress within the scaffold in a particular area and optimize input flow rate consequently.

7.2 Application of gold nanoparticles as contrast agents in confocal laser scanning microscopy

Noble metal nanoparticles, in particular gold nanoparticles, have a number of promises in biomedical optics due to their outstanding physical properties (Jain *et al.*, 2007; Khlebtsov, 2008). They have been used for centuries, for example in Roman items and in stained glass windows. Nowadays, gold nanoparticles play an important role in a range of fields, including material sciences, engineering, biology, and medicine. Illuminated by laser radiation at the specific wavelength, gold nanoparticles present a strong enhancement of scattering and absorption of the electromagnetic field. This phenomenon, called plasmon resonance, relies on the coherent oscillation of conductive electrons (Maier, 2007). The plasmon resonance occurs in a narrow range of wavelengths depending on the size of the nanoparticles and the dielectric properties of the surrounding medium (Maier, 2007; Prasad, 2004).

The shape of nanoparticles is also an important parameter that can deeply modify the plasmon resonance frequency (Oldenburg *et al.*, 1998; Hirsch *et al.*, 2006; Murphy *et al.*, 2005; Perez-Juste *et al.*, 2005). Gold nano-shells, for example, can resonate across a broad range of wavelengths (Oldenburg *et al.*, 1998; Hirsch *et al.*, 2006). These particles are composed of silica core surrounded by gold nano-shell, or by other noble metal shells. The variations of their relative core and shell thicknesses enable tuning the plasmon resonance from visible to infrared spectral area (Oldenburg *et al.*, 1998; Hirsch *et al.*, 2006).

A similar effect is obtained with gold nano-rods: these rodshaped particles display two plasmon resonance peaks, related to the long and short axes of the particle that defining the relative position of the plasmon frequencies (Murphy *et al.*, 2005; Perez-Juste *et al.*, 2005).

The gold nanoparticles are potentially interesting to be used as a contrast agent in cancer diagnostics and therapy (Loo *et al.*, 2004). Their application as a contrast agent helps in creating differences between various tissue components as well as in differentiating cancerous from healthy tissues (Loo *et al.*, 2004; Kah *et al.*, 2008; Sokolov *et al.*, 2003; El-Sayed *et al.*, 2005). Whereas, commonly employed fluorescent contrast agents, such as fluorescent dyes and proteins, suffer from loss of fluorescence intensity with time (i.e. photobleaching) (Mycek and Pogue, 2003). Besides, the natural fluorescence of tissues, also known as autofluorescence, is often quantitatively comparable with the signal produced by fluorescent contrast agents (Mycek and Pogue, 2003). Nanoparticles, on the contrary, are not subject to photo-bleaching and are also biocompatible. Furthermore, the modification and bioconjugation with biomolecules (Kah *et al.*, 2008; Sokolov *et al.*, 2003; El-Sayed *et al.*, 2005) allows targeting specific cells (Tanev *et al.*, 2006). In addition, the enhancement of electromagnetic field generated by nanoparticles during plasmon resonance provides a strong signal that overpasses the autofluorescence.

This is especially achievable in the near-infrared spectral region where the tissues autofluorescence is minimal (Frangioni, 2003; Churmakov *et al.*, 2004). Thus, gold nanoparticles have a great potential applying as a contrast agent with optical imaging modalities, such as a confocal laser scanning microscopy (CLSM).

Contrariwise, instead of contrast agents mentioned above the so-called optical clearing agents, such as glycerol or propylene glycol, have been widely applied to improve image contrast and increase depth of image probing (Tuchin *et al.*, 1997; Proskurin and Meglinski, 2007; Vargas *et al.*, 1999; Vargas *et al.*, 2001). It has been concluded that matching of the refractive indices of the structural elements of tissue cells is caused, that produces a temporal reduction of light scattering and increases the transparency of superficial tissues (Tuchin, 2005).

7.2.1 Optical properties of gold nanoparticles

In 1908, Mie suggested a solution describing the interaction of an electromagnetic wave with small conductive spherical particles (van de Hulst, 1957). With appropriate boundary conditions for spherical objects this solution is presented as a series of multipole oscillations and the extinction crosssection of nanoparticles can be presented as:

$$\sigma_{ext} = \frac{2\pi}{|\mathbf{k}|^2} \sum_{L=1}^{\infty} (2L+1) \text{Re}(a_L + b_L). \quad (7.1)$$

Respectively, scattering and absorption cross-sections are defined as:

$$\sigma_{sca} = \frac{2\pi}{|\mathbf{k}|^2} \sum_{L=1}^{\infty} (2L+1) (|a_L|^2 + |b_L|^2), \quad (7.2)$$

$$\sigma_{abs} = \sigma_{ext} - \sigma_{sca}.$$

Here, $\mathbf{k}$ is the wave-vector, L is the term index, corresponding to the dipole oscillation (if $L = 1$), to the quadrupole oscillation (if $L = 2$),

$$\begin{aligned} a_L &= \frac{m\psi_L(mx)\psi'_L(x) - \psi'_L(mx)\psi_L(x)}{m\psi_L(mx)\eta'_L(x) - \psi'_L(mx)\eta_L(x)}, \\ b_L &= \frac{\psi_L(mx)\psi'_L(x) - \psi'_L(mx)\psi_L(x)}{\psi_L(mx)\eta'_L(x) - m\psi'_L(mx)\eta_L(x)}. \end{aligned} \quad (7.3)$$

Here, $m = n/n_m$, n and n_m are the refractive indices of particle and surrounding dielectric medium, respectively; ψ_L and η_L are the Ricatti-Bessel cylindrical functions, $x = |\mathbf{k}|r$, r is the radius of the nanoparticle. The scattering and absorption properties of spherical nanoparticles differ with size: for particles with a diameter larger than the wavelength of

incident radiation λ ($d > \lambda/20, d = 2r$) the cross-section can effectively be decomposed as a superposition of oscillating electric dipoles, quadrupoles, and higher-order multipoles; for particles with a diameter smaller than the wavelength of incident radiation ($d < \lambda/20$) σ_{ext} can be described as an electric dipole. The Rayleigh theory well describes the latter case and is sufficient to understand the plasmon resonance. Rayleigh assumed that all of the electrons oscillate with the same phase and frequency as the incident wave, and the particle behaves as a large oscillating dipole moment (known as a dipole approximation) (Maier, 2007; Prasad, 2004).

The expressions for (7.1) and (7.2) can be re-written then in the form:

$$\begin{aligned}\sigma_{ext} &= 9 \frac{\omega}{c} (\varepsilon_{diel})^{\frac{3}{2}} V \frac{\varepsilon_{met}''}{(\varepsilon_{met}' + 2\varepsilon_{diel})^2 + (\varepsilon_{met}'')^2}, \\ \sigma_{sca} &= \frac{3}{2\pi} \left(\frac{\omega}{c}\right)^4 (\varepsilon_{diel})^2 V^2 \frac{(\varepsilon_{met}' - \varepsilon_{diel})^2 + (\varepsilon_{met}'')^2}{(\varepsilon_{met}' + 2\varepsilon_{diel})^2 + (\varepsilon_{met}'')^2}.\end{aligned}\tag{7.4}$$

Here, V is the volume of the particle, ω is the angular frequency of incident light, and c is the speed of the light in a vacuum; ε_{diel} is the dielectric constant of the surrounding medium and ε_{met} is the wavelength-dependent complex dielectric function of the metal nanoparticles, $\varepsilon_{met} = \varepsilon_{met}' + i\varepsilon_{met}''$. When ε_{met}'' is dependent weakly on ω the maximum of σ_{ext} is corresponding to the resonance condition: $\varepsilon_{met}' = -2\varepsilon_{diel}$ and the denominator in (7.4) tends to minimum. If this condition is fulfilled the plasmon resonance (Fig. 7.5) appears at $\sim 520 \text{ nm}$ for gold nanoparticles (Kreibig and Vollmer, 1995).

7.2.2 Description of the experiment

The CLSM (510 Invert Laser Scan Microscope, Carl Zeiss, Germany) was employed for imaging of skin tissue samples. The CLSM (Sheppard *et al.*, 1997) offers several advantages over conventional fluorescence-based microscopy, including the ability to control depth of field, elimination or reduction of background information away from the

focal plane, and the capability to collect serial optical sections from thick specimens (Sheppard *et al.*, 1997). In CLSM a laser beam is directed by a dichroic mirror towards a pair of mirrors that perform samples scanning in x and y directions (Meyer and Lademann, 2007; Martschick *et al.*, 2007; Lademann *et al.*, 2008a; Lademann *et al.*, 2008b). The laser beam then passes through the microscope objective and excites the fluorescence within the sample. Subsequently, the excited fluorescent radiation goes back through the objective, dichroic mirror and a pinhole, which is placed in the conjugate focal plane (Fig. 7.6).

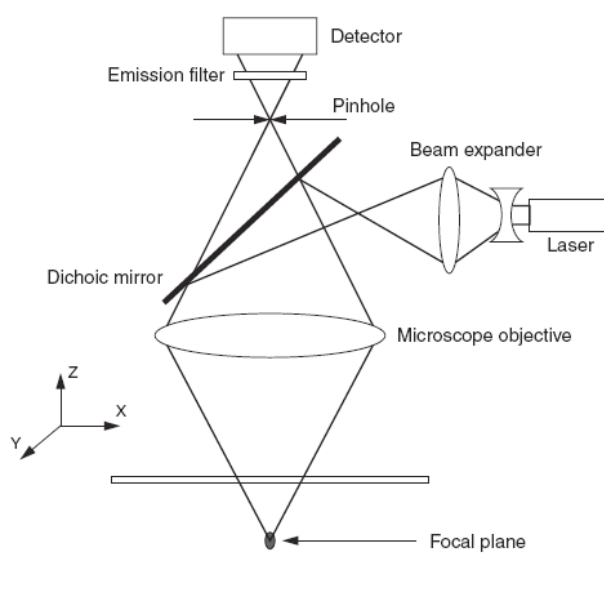


Figure 7.6. Schematic diagram of a confocal microscope used in the current research.

The pinhole serves to exclude fluorescence from the region outside the focal plane; since only the signal from the focal plane is detected and high quality image is obtained. The CLSM system is equipped with an argon-krypton laser at the excitation wavelength 543 nm.

Chicken skin was used as an object of interest. The solution of glycerol and gold nano-spheres (concentration of 109 *particles/mL*) were employed as contrast agents. The gold nano-colloids (British Biocell International, Cardiff, UK) with an average size of 60 *nm* in diameter were diluted with distilled water. The skin sample was observed every 5 *min* during the diffusion of these contrast agents within the tissue, 200 pictures spaced by 450 *nm* taken at the exactly same conditions.

7.2.3 Results

The image of chicken skin (1 cm^2) has been obtained using a 63x oil immersion objective. The details of subsurface tissue structure are seeing in some way before application of glycerol (see Figure 7.7-a), however, already at a depth of 45 μm picture is not clear (see Figure 7.7-b).

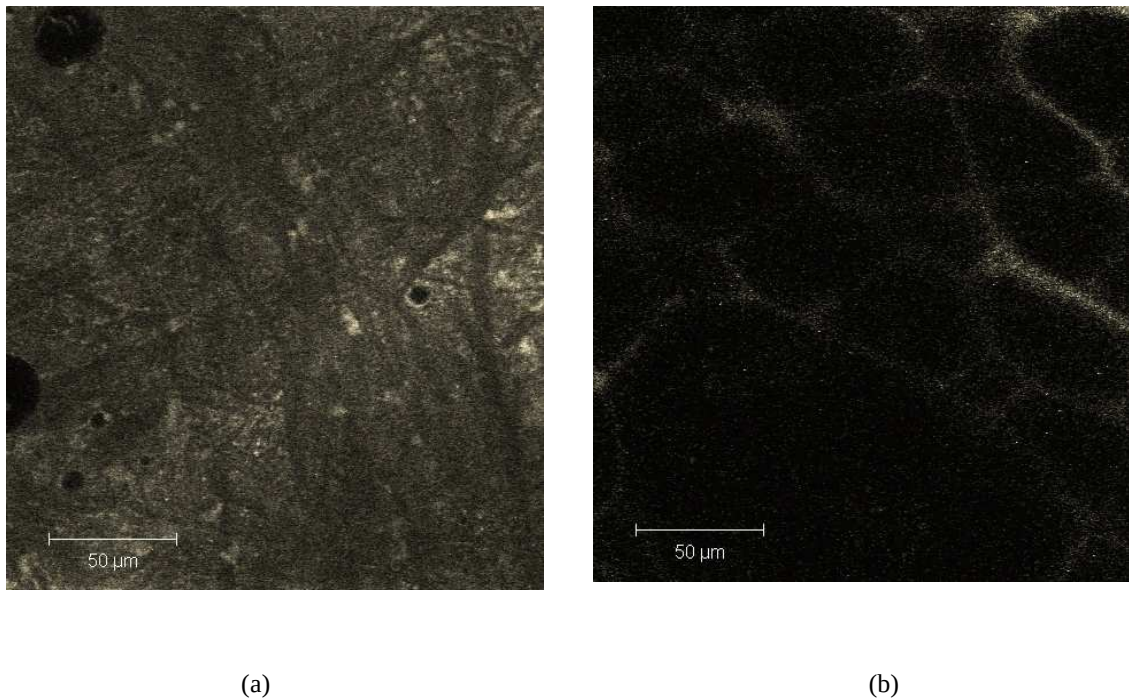


Figure 7.7. CLSM images of chicken skin obtained at the focal depth 18 μm (a) and 45 μm (b) at normal conditions.

Similar experiment was carried out with 60 nm gold nano-spheres used as a contrast agent with concentration 109 particles/mL (Figure 7.8). The improvement of the image contrast is observed after 25 min after using nanospheres (see Fig.7.8-a), and maximum enhancement of image contrast observed after 60 min (see Figure 7.8-b). Both images are obtained at a depth of 45 μm .

The use of glycerol reduces the effects of multiple scattering (Meglinski *et al.*, 2002; Tuchin, 2005), which significantly improves the quality of CLSM images after 15 minutes after application (Fig.7.9-a and 7.9-b, the focal plane at 18 μm and 45 μm , respectively).

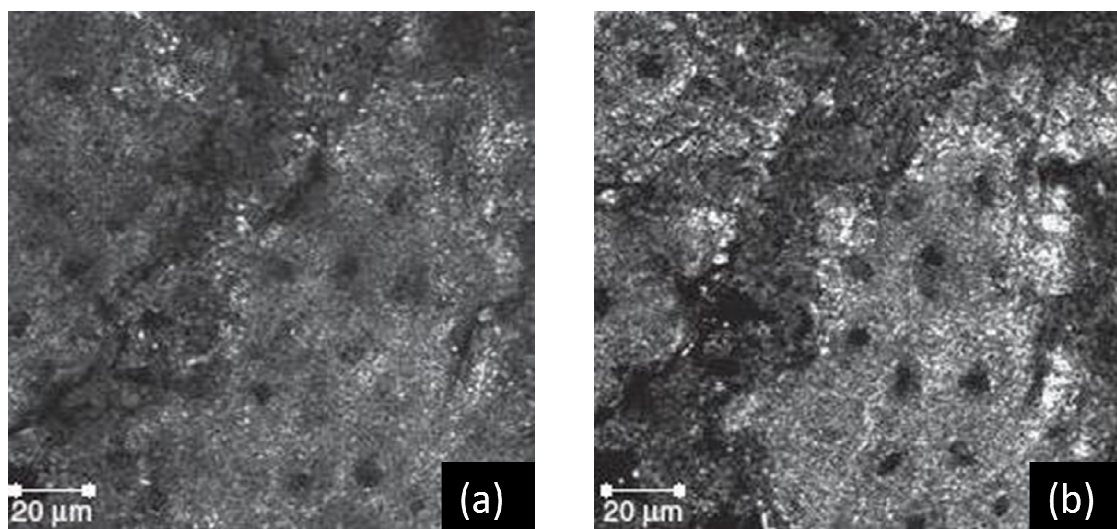


Figure 7.8. CLSM image of chicken skin obtained at 45 μm focal depth after 25 (a) and 60 (b) minutes diffusion of 60 nm gold nano-spheres into the skin sample.

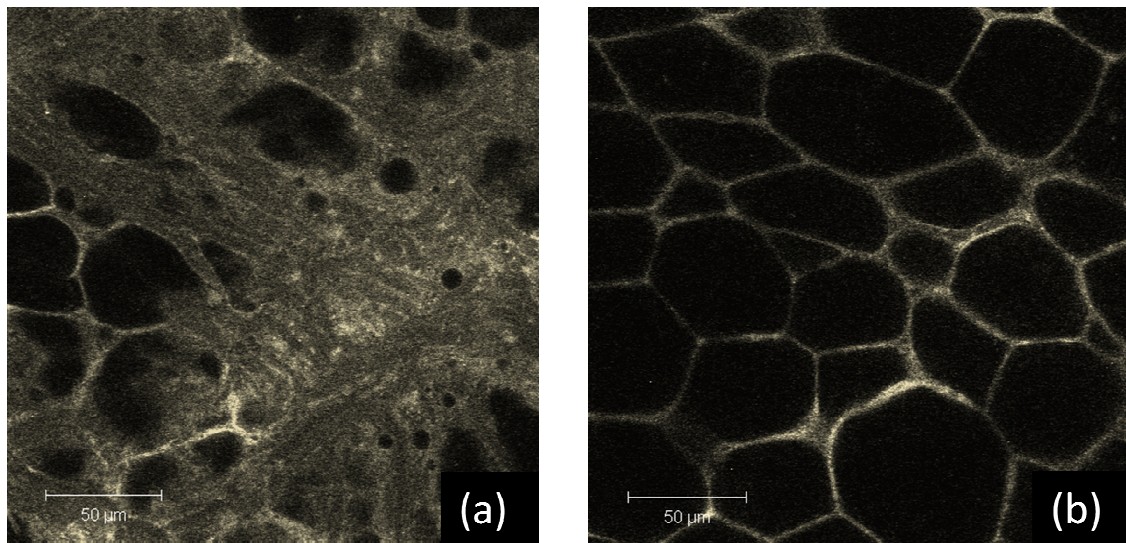


Figure 7.9. CLSM images of chicken skin with application of clearing agent – glycerol: (a) – focal plane at 18 μm ; (b) – focal plane at 45 μm .

These results demonstrate that the use of glycerol as a contrast agent is an effective way to improve the CLSM images. However, contrasting with the help of glycerol is not tissue-specific. Nevertheless, in our opinion, the use of nanoparticles labelled with biomarkers in combination with osmotic active immersion liquids might be preferable. In addition, the increased concentration of nanoparticles, as well as using of their aggregates, could significantly increase the contrast of the image of individual structural elements in biotissues.

Conclusions

In the current Ph.D. thesis MC technique was applied for the purposes of simulation of OCT. The MC simulation makes it possible to give a realistic picture of the processes involved into the light-tissue interaction. Although the modelling of optical propagation within biotissues with the MC is relatively time consuming, it is the most efficient technique to predict and analyze the peculiarities of the radiation propagation in the complex turbid medium. Existing MC code was improved with coherence and polarization effects, which significantly increasing the accuracy of the modelling. MC model was used also for the purpose of visualization of the inhomogeneity in turbid medium with the help of artificial neural network (Kovaleva *et al.*, 2007). Low coherence optical techniques, such as OCT, its extension Doppler OCT and CLSM were improved and applied for the purposes of bio-tissues diagnostics and imaging of their internal structure. In addition CLSM was employed for the investigations of image contrasting with gold nanoparticles (Lemelle *et al.*, 2009).

MC model was developed and improved (see Ch.6) for adequate simulation of the non-invasive low coherence optical diagnostic which is a novel modern technique uniquely suited for the measurements of particles distribution within the topical layers of biological tissues and other complex media, such as polymers, colloids, composite materials, etc. This approach is based on the illumination of a medium by optical radiation with a low coherence both spatial and temporal, and analysis of the back-scattering signal. Recently introduced this technique is not currently used. The aim of the research was the development of the theoretical model of low coherent radiation propagation and localization within the random media and establishment ultimate limits and optimal instrument parameters of the experimental system suitable for the biomedical diagnostics. The proposed technique is likely lead to cancer diagnostics and quantitative monitoring of structure of complex composite materials. The MC method makes it possible to give a realistic picture of the processes involved into the light-tissue interaction. Although the modelling with the MC is quite time consuming, it is the most efficient technique to

describe the radiation propagation in the scattering turbid medium. The main difficulty in the applying of the low coherence technique in biomedical diagnostics is the complexity of biotissues structure. For example, the human skin consists of the five layers with unique optical properties in each layer. External boundary and internal boundaries between layers are rough and wavy. However, with the proposed MC technique it was possible to use the multilayered model of skin which consists of 7 layers (see Sec.6.1). Each layer has its own optical properties, such as μ_s – scattering coefficient, μ_a – absorption coefficient, g – anisotropy factor and n – refractive index. The sampling volume was simulated for various probe geometry. The results of the simulation clearly show that probe with separated source-detector field of view significantly improve the localization of detected signal within the medium.

Proposed MC model was expanded with the new computational method that is able to consider all the scattering orders within a detected OCT signal (see Sec. 6.2). The method is based on the iterative procedure of the solution of Bethe-Salpeter equation. The new approach is able adequately account for the ballistic component of the detected OCT signal including the contribution of the rear interfaces within OCT signal. These will increase the resolution of the simulated OCT signal.

Based on the generalization of iterative procedure of the solution of Bethe-Salpeter equation, a new vector-based MC approach has been developed (see Sec.6.3). The performances of the developed method are shown both for conventional and polarization-sensitive OCT modalities. The current approach allows considerably simplified simulation of polarized low-coherent optical radiation propagation within turbid scattering media avoiding implementation of Stokes-Mueller and/or Jones formalism, with the substantial reducing of the computational resources. In a similar manner this approach can be applied for circular polarization allowing one to avoid a cumbersome calculation of the Mueller matrix. The technique can provide detailed information on polarization propagation within/outside the medium, scoring various physical quantities simultaneously. The technique will find a number of straightforward applications related to the non-invasive

diagnostics of various disperse random media, including biological tissues, polymers, liquid crystals, and others.

Based on the results of MC simulations the OCT set-up was developed and improved. The conventional OCT was applied for the purpose of scaffold structure imaging (see Sec.7.1). Three-dimensional porous scaffolds are used for the directional growth of the cells and for the supplying them with nutrients in tissue engineering. At the same time Doppler OCT was applied for the measurements of spatial fluid flow velocity distribution within this complex scaffold structure. Obtained results make it possible to evaluate the shear stress within the scaffold in a particular area and optimize input flow rate consequently.

In framework of optical tomography and related problems it is required to solve so-called inverse problem. This includes reconstruction of spatial positioning of the absorbing and/or scattering macro-inhomogeneities distribution within the medium by the characteristics of scattered radiation. This distribution depends on the distance between photo-detector and the entrance point of laser beam, and is determined by the scattering properties of the biological tissue. To describe the propagation of laser radiation within the complex random media conventionally statistical computational Monte Carlo (MC) method, which combines the statistical weight scheme and effective optical photon paths simulation, was used (see Sec.4.4). It is assumed that inhomogeneity located in subsurface area of the medium changes the intensity distribution of the back-scattered radiation. This makes it possible to apply artificial neural networks (ANN) for finding the dimensions and locating of the inhomogeneity. This is especially of high priority because of new diagnostics/treatment developing that is based on the applying gold nanoparticles for labelling cancer cells.

As an addition the influence of gold nanoparticles on the resolution of image in optical imaging was studied in the current research (see Sec.7.2). The use of nanoparticles labelled with biomarkers in combination with osmotic active immersion liquids might be preferable and have a great potential applying as a contrast agent with optical imaging modalities, such as a confocal laser scanning microscopy (CLSM). The increased concentration of

nanoparticles, as well as using of their aggregates, could significantly increase the contrast of the image of individual structural elements in biotissues.

The obtained results of the whole research in my Ph.D. thesis show the feasibility of using low-coherence optical diagnostic techniques, such as OCT and CLSM, for the purposes of diagnostics and treatment of biotissues. MC modelling can serve as an instrument of optimization and adjustment of the applied optical techniques. Preferability of using of these techniques, especially OCT and Doppler OCT, is confirming with relatively low cost of building such instruments in comparison with cost of traditional MRI. Hence, from the financial point of view return on invested money will be much higher in case of OCT and Doppler OCT, which make them more attractive for the usage in commercial projects.

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